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PROCEEDINGS
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A NEW DISCUSSION OF THE CHANGES IN THE EARTH'S RATE OF ROTATION

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Read before the Academy, November 6, 1951

1. Three types of changes in the rotation of the earth are known to exist: (1) a gradual lengthening of the day; (2) irregular fluctuations in the rate of rotation, the accumulated effect of which has amounted to approximately ±30 sec. during the past three hundred years; (3) seasonal changes in the rate of rotation, causing the earth's rotation to be ahead by about $0^{s}06$ in November and behind by nearly the same amount in May. The existence of this type of change was first discovered by N. Stoyko[1] in 1937, and has been confirmed by results obtained in the Greenwich Time Service.[2]

G. I. Taylor[3] and Harold Jeffreys[4] showed that tidal friction in shallow seas is adequate to account for the rate at which the length of the day appears to have increased since the date of the most ancient astronomical records available.

W. H. Munk and R. L. Miller[5] showed that seasonal fluctuations in atmospheric and oceanic circulation are of the required order of magnitude to explain the observed changes with an annual period.

The explanation of the irregular fluctuations has been more elusive. W. de Sitter[6] represented the observed fluctuation curve by a series of straight lines. This representation requires abrupt changes in the rate of rotation of the earth. Sir Harold Spencer Jones showed that more accurate data than de Sitter had available did not confirm de Sitter's representation in detail, and that this representation could only be considered a rough approximation.

This is one of several aspects of the problem that remained in doubt until the two important contributions by Spencer Jones in 1932[7] and 1939.[8] In the former of these papers Newcomb's discussion of occultations of stars by the moon was revised. These results added significantly to our present knowledge of the fluctuation curve. In the latter paper it was clearly established that the fluctuations in the mean longitudes of the sun, Mercury

and Venus correspond to fluctuations in time identical with those corresponding to the fluctuations in the moon's mean longitude. It was also shown in this paper that if for the secular acceleration term in the moon's mean longitude the value obtained from a discussion of ancient observations,[4] + 5".22 T^2, is adopted (T expressed in centuries), then the modern observations of the sun and the inner planets require a term + 1".23 T^2

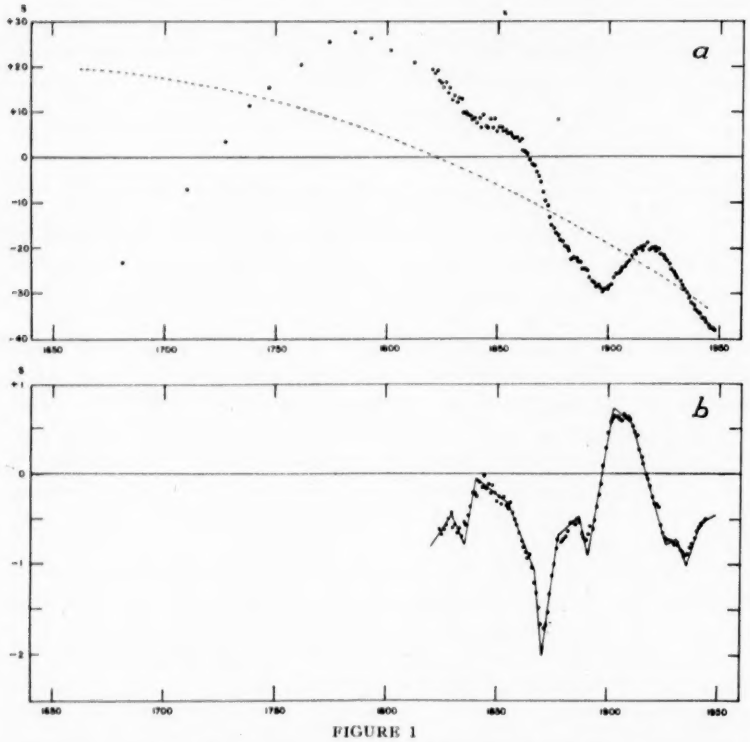

FIGURE 1

Upper curve; fluctuation curve in the rotation of the earth; the dashed line represents the parabolic solution. Lower curve: the dots represent the derivatives obtained from a nine-point formula, the straight lines the derivatives of parabolic arcs fitted to the fluctuation curve.

in the sun's mean longitude. In the mean longitudes of the planets the coefficient equals that for the sun multiplied by the ratio of the mean motions of the planets to that of the sun. These ratios are 4.152 for Mercury, 1.626 for Venus. An exhaustive discussion of Mercury by G. M. Clemence[9] confirmed Spencer Jones's conclusion, both as to the ratio of the fluctu-

ations in seconds of arc in the mean longitude of Mercury to those in the moon and as to the coefficient of T^2.

Since 1820 annual values of the fluctuation curve are available. The accuracy increases considerably about 1850, 1880 and again in 1923 when E. W. Brown's new tables were introduced for the calculation of the lunar ephemeris.

The dots in figure 1 (*b*) give the derivative of the fluctuation curve in the earth's rotation obtained by fitting by least squares solutions parabolic arcs through each nine consecutive annual values of the fluctuation and taking the derivative for each mid-point. These derivatives can be approximate only due to the rounding inherent in the nine-point formula. However, a straight line character of the derivative is suggested. By successive ap-

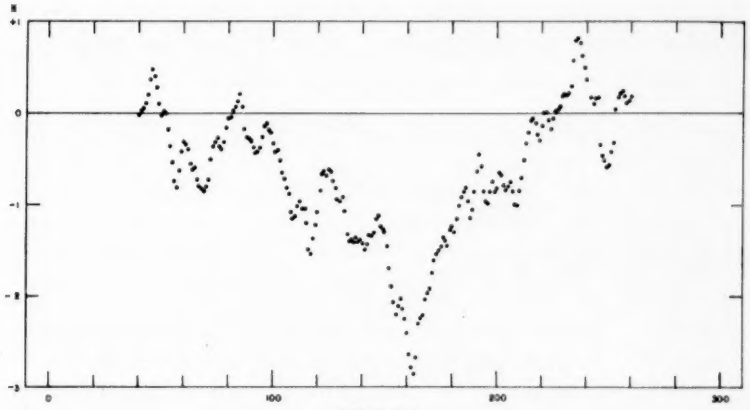

FIGURE 2

Cumulative effects of random deviations, normal distribution $\sigma = 0.15$.

proximations parabolic arcs were then fitted to the fluctuation curve over intervals for which the derivative appeared to be nearly a straight line. The derivative of the parabolic sections curve is represented by the straight lines in the same figure. The fluctuation curve is remarkably well represented by this series of parabolic arcs.

In the course of a further study of this problem I was led to examine whether the apparent straight-line character of the derivative of the fluctuation curve could be accounted for by postulating random cumulative changes in the length of the year. If $F(k)$ represents the fluctuation curve in seconds of time for successive years, the differences should then be represented as in the table below,

$$\begin{array}{ll} F(0) & \\ & \Delta \\ F(1) & \\ & \Delta + \delta_1 \\ F(2) & \\ & \Delta + \delta_1 + \delta_2 \\ F(3) & \\ & \Delta + \delta_1 + \delta_2 + \delta_3 \\ F(4) & \end{array}$$

the δ's having a mean value zero and standard deviation σ to be obtained from a discussion of the observational data.

Dr. A. J. J. van Woerkom and I examined extensive samples of accumulations of random numbers, a selection of which is given in figure 2. The similarity with the derivative of the fluctuation curve is sufficiently striking to suggest that the hypothesis may be compatible with the fluctuation phenomenon.

2. If the proposed interpretation of the fluctuations in the earth's rate of rotation is correct, then the sum of the random increments δ after n years should have a root mean square value $n^{1/2}\sigma$. In the fluctuation itself,

$$F(n) = F(0) + n\Delta + (n-1)\delta_1 + (n-2)\delta_2 + \ldots + \delta_{n-1};$$

the root mean square value of the double accumulation of the δ's is given by

$$\sigma_n^2 = \left(\frac{n^3}{3} - \frac{n^2}{2} + \frac{n}{6}\right)\sigma^2.$$

For large values of n

$$\sigma_n \simeq 3^{-1/2} n^{3/2} \sigma.$$

Thus $F(n) - F(0) - n\Delta$ may be expected to have an amplitude proportional to $|T|^{3/2}$, if T is the time in centuries counted from any epoch.

A solution was made from the values of the fluctuations derived from the observed motion of the moon during the last three centuries and from records of eclipses back to 720 B.C. For the data before 1681 I used the residuals furnished by Simon Newcomb,[10] corrected to correspond to the system that was adopted by Spencer Jones[7] in his latest discussions. This corresponds to taking the moon's mean longitude from Brown's tables corrected by

$$\Delta L_\mathfrak{C} = +22''.83 + 23''.40T + 5''.22T^2 - 10''.71 \sin (140°T + 20°.7),$$

the last term of which eliminates the empirical term present in the lunar tables. The correction to the sun's mean longitude from Newcomb's tables is

$$\Delta L_\odot = + 5''.20 + 5''.43T + 1''.23T^2.$$

In these and all following expressions T is counted in centuries from 2000 A.D.

Modern observations alone do not permit the evaluation of the T^2 terms. If they are to be modified, the following changes must be made, as stated by Spencer Jones[8]:

$$\text{in the moon's longitude, } +5''.22T^2 \rightarrow (+5''.22 + s)T^2;$$
$$\text{in the sun's longitude, } +1''.23T^2 \rightarrow (+1''.23 + .0748s)T^2,$$

the factor 0.0748 being the ratio between the mean motions of the sun and the moon. In order to agree with the modern observations the fluctuation in the moon's mean longitude must be changed correspondingly, $B \rightarrow B - sT^2$.

The solution was made by the method of least squares from the observation equations

$$x + Ty + T^2z = F,$$

F being the fluctuation in seconds of time in the earth's rotation, related to the fluctuation B in the moon's mean longitude by

$$F = 1.821\ B.$$

The choice of zero point of T, 55 years beyond the date of the latest equation used, is arbitrary. Choosing the zero date too near a date for which an equation is available is undesirable for obvious numerical reasons. The solution is to be made on the assumption that the mean error of F increases with $(-T)^{3/2}$. Hence the equations were multiplied by the factor $(-T)^{-3/2}$, and the right-hand members of the new equations were treated as having equal mean errors. The coefficients and right-hand members of the equations solved,

$$ax + by + cz = l,$$
$$a = (-T)^{-3/2}, \quad b = (-T)^{-1/2}, \quad c = (-T)^{+1/2}, \quad l = F(-T)^{-3/2},$$

are given in the table. The solution obtained is

$$x = -54^s.6 \pm 0.544\ \mu^*,$$
$$y = -40.9 \pm 0.645\ \mu^*,$$
$$z = -5.46 \pm 0.109\ \mu^*,$$

in which μ^* is the mean error of the right-hand members l of the observation equations. (Throughout this paper the mean error will be used to indicate the uncertainty of a numerical result.) The table gives the residuals v^* of the l-equations as well as the residual fluctuations F^*.

The mean error obtained from the residuals v^* is $\pm\ 6^s.8$. While it is permissible to take μ^* equal to this value to indicate the accuracy with which the past record of the earth's rotation is represented, other aspects of the problem require further discussion. The reason is that the random process may produce a spurious quadratic term.

3. In order to solve this and several related problems that arose in this investigation, Dr. van Woerkom and I constructed artificial fluctuation curves with initial values $F(0) = 0$, $\Delta = 0$ from a sequence of random δ's with mean value zero and a normal distribution. Equations were then solved with the same left-hand members as the equations in table 1, but with the right-hand members taken from the artificial fluctuation curves

TABLE 1

EQUATIONS USED FOR THE PARABOLIC SOLUTION FROM FLUCTUATIONS IN THE EARTH'S ROTATION

YEAR	T	F	a	b	c	l	v^*	F^*
−683	−26.83	−661″	0.00719	−0.1929	5.176	−4°.8	+16°.0	+2327″
−380	−23.80	−2051	0.00861	−0.2049	4.877	−17.7	+1.0	+202
−189	−21.89	−1472	0.00977	−0.2139	4.682	−14.4	+2.9	+370
+135	−18.65	−1339	0.01242	−0.2313	4.314	−16.6	−1.8	−100
850	−11.50	−517	0.0256	−0.2944	3.386	−13.2	−5.4	−192
927	−10.73	−297	0.0284	−0.3047	3.269	−8.4	−1.5	−37
986	−10.14	−617	0.0310	−0.3143	3.187	−19.1	−12.9	−401
1621	−3.79	+42	0.136	−0.5154	1.953	+5.7	+2.7	+22
1635	−3.65	−5	0.143	−0.5220	1.905	−0.7	−3.8	−25
1639	−3.61	−53	0.146	−0.5271	1.903	−7.7	−10.9	−73
1645	−3.55	−22	0.149	−0.5290	1.878	−3.3	−6.6	−42
1653	−3.47	−38	0.155	−0.5378	1.866	−6.9	−9.3	−58
1662	−3.38	−27	0.161	−0.5442	1.839	−4.3	−7.7	−47
1681	−3.19	−23.1	0.176	−0.5614	1.791	−4.1	−7.7	−42.1
1710	−2.90	−7.1	0.202	−0.5858	1.699	−1.4	−5.1	−24.1
1727	−2.73	+3.5	0.222	−0.6061	1.655	+0.8	−2.8	−11.9
1738	−2.62	+11.3	0.236	−0.6183	1.620	+2.7	−0.9	−2.9
1747	−2.53	+15.8	0.249	−0.6300	1.594	+3.9	+0.4	+2.7
1760.9	−2.391	+20.4	0.270	−0.6456	1.544	+5.5	+2.2	+9.1
1774.1	−2.259	+25.5	0.295	−0.6664	1.505	+5.0	+2.1	+16.2
1785.5	−2.145	+27.5	0.318	−0.6821	1.463	+8.7	+6.1	+20.1
1792.6	−2.074	+26.2	0.335	−0.6948	1.441	+8.8	+6.5	+20.0
1801.8	−1.982	+23.5	0.358	−0.7096	1.406	+8.4	+6.6	+19.0
1811.9	−1.881	+20.8	0.388	−0.7298	1.373	+8.1	+6.9	+18.2
1825	−1.750	+15.9	0.432	−0.7560	1.323	+6.9	+6.8	+16.0
1835	−1.650	+9.9	0.472	−0.7788	1.285	+4.7	+5.6	+12.2
1845	−1.550	+6.7	0.518	−0.8029	1.244	+3.5	+5.7	+11.3
1855	−1.450	+5.4	0.573	−0.8308	1.205	+3.1	+7.0	+12.4
1865	−1.350	−1.0	0.637	−0.8600	1.161	−0.6	+5.3	+8.5
1875	−1.250	−15.4	0.715	−0.8938	1.117	−11.0	−2.4	−3.2
1885	−1.150	−22.2	0.811	−0.9326	1.072	−18.0	−6.0	−7.3
1895	−1.050	−28.1	0.929	−0.9754	1.024	−26.1	−9.7	−10.3
1905	−0.950	−25.6	1.080	−1.0260	0.975	−27.6	−5.3	−4.9
1915	−0.850	−19.2	1.276	−1.0846	0.922	−24.5	+5.8	+4.6
1925	−0.750	−21.9	1.540	−1.1550	0.866	−33.7	+7.8	+5.1
1935	−0.650	−29.5	1.908	−1.2402	0.806	−56.3	+1.5	+0.8
1945	−0.550	−36.6	2.452	−1.3486	0.742	−89.7	−7.0	−2.9

for the same values of T in terms of 100 steps, only the sign of T being reversed. From solutions based on fifteen independent artificial fluctuation sequences it was found that the root mean square value of $FT^{-3/2}$ is 5.3 ± 3.1 times the root mean square value of the residuals after substitution of the quadratic solution. Thus the standard deviation of v^* multiplied by 5.3 gives the root mean square value of the accumulation after 100 years,

$\mu = 36^s \pm 21^s$. The standard deviation of the random change in the length of the year that corresponds to this result is $\sigma = 0^s063 \pm 0^s037$.

A second method of evaluating σ is from the derivatives for the last 129 years of the fluctuation curve in the earth's rotation. For 25 dates with an interval of 5 years the values of the derivative $\dot{F}$, with unit of time one year, were taken from both the nine-point formula and from the straight-line representation. From these the derivatives $\dot{F}^*$, corrected for the quadratic solution, were obtained by $100\dot{F}^* = 100\dot{F} - y - 2zT$. A least squares solution from the 25 equations

$$\sigma^{*2} = \frac{\dot{F}^{*2}}{-100T}$$

gave for the two solutions $\sigma^* = 0^s053$ and 0^s054, respectively. Again, this is a standard deviation reduced by the elimination of the quadratic solution. Comparisons with the artificial fluctuation curves gave for this case the ratio $\sigma/\sigma^* = 2.2 \pm 1.2$. The derivatives therefore lead to a standard deviation of the random cumulative changes in the length of the year, $\sigma = 0^s12 \pm 0^s06$. Although the formal uncertainty of this determination is greater than that obtained from the fluctuation curve, the two values may be entitled to nearly equal weight. Hence $\sigma = 0^s09 \pm 0^s03$ may be an acceptable compromise. A value of $\sigma = 0^s12$ is, however, not excluded. The appearance of the derivative curve favors this larger value.

4. The coefficient z of T^2 obtained in the solution may be considered to be made up of two parts: $z = z_\tau + z_a$, z_τ being due to the tidal retardation of the earth's rotation, z_a the spurious effect introduced by the accumulation of the random process. Only the sum of the two parts is obtained from observation. It is possible, however, to give the root mean square value σ_z of z_a.

Let the earliest date used in the solution be denoted by $-T_e$. From the representation

$$F(T_e) = x + yT_e + zT_e^2 + F^*(T_e),$$

it can be shown without difficulty that the root mean square value of z, determined from fluctuation sequences with $F(0) = 0$, $\Delta = 0$, is almost exclusively determined by $F(T_e)$. Thus, approximately,

$$\sigma_z T_e^2 = \frac{1000}{\sqrt{3}} T_e^{3/2}\sigma,$$

the right-hand member of which is the root mean square value of the accumulation in F after $100\,T_e$ steps, or

$$\sigma_z = \frac{1000}{\sqrt{3T_e}}\sigma.$$

With $T_4 = 26.83$, it follows that with

$$\sigma = \pm 0^s09, \qquad \sigma_z = \pm 10^s0;$$
$$\sigma = \pm 0^s12, \qquad \sigma_z = \pm 13^s3.$$

The value of σ_z was confirmed by evaluations of z from the fifteen artificial sequences. It was then also found that there is no significant relation between the value of z obtained in the quadratic solution from a fluctuation sequence and the factor by which the root mean square value of $FT^{-3/2}$ is reduced by the substitution of the quadratic solution.

The proper interpretation of the result for z is, therefore,

$$z_r = -5^s46 \pm \sigma_z \pm 0.109\,\mu^*.$$

If $\sigma_z = 10^s0$, $\mu^* = 6^s8$,

$$z_r = -5^s46 \pm 10^s0,$$

the contribution due to μ^* being insignificant. The correction to the coefficient of T^2 in the moon's mean longitude becomes $s_r = -3''.00 \pm 5''.5$. Further comments on this result will be found in section 7.

5. The large corrections found call for further examination. When Newcomb made his solution, he solved for five unknowns, three of which correspond to x, y, z of the present discussion. The two additional unknowns were to define the amplitude and phase of a sine curve with period of 275 years. A provisional discussion had yielded this period as in best agreement with the empirical representation of the fluctuation curve during the last three centuries. Solving for the five unknowns, Newcomb found $s = -0''.47$. If his equations are solved with x, y, z as the only unknowns, the solution gives $s = -3''.30$. Newcomb's data are not immediately comparable with those used in the present discussion, and in the choice of the ancient observational data his opinion differed from that of others who discussed these records. The change of about $-3''$ in the solution brought about by omitting the unknowns connected with the empirical term with period 275 years is, however, significant.

Newcomb's solution was adopted by E. W. Brown with slight modifications in the empirical term and in x, y, z. In Fotheringham's evaluation of the coefficient of T^2 in the moon's longitude from ancient observations, the moon's mean motion at 1800 or 1900 was taken to correspond to Brown's formulae, again with minor modifications. The system in which the fluctuations B in the moon's mean longitude have been reckoned has thus resulted from an attempt to represent the last three centuries by a sine curve.

If the fluctuation is to be considered the result of random cumulative changes in the length of the year, then the 275-year term has no real meaning, and a revised solution without the two additional unknowns to allow for an adjustment of amplitude and phase of this spurious term is to be pre-

ferred. It is this modification rather than the new weighting of the older observations that has produced the large changes in the solution.

The solution obtained must be considered a provisional result. I used the same selection of the older observations as had been used by Newcomb because they were available in a suitable form. A new solution which I hope to undertake in collaboration with Dr. E. W. Woolard of the U.S. Naval Observatory should include all of the available older observations from the earliest records to the time of the invention of the telescope. A new reduction of these observations directly with Brown's tables for the moon and Newcomb's tables for the sun will be desirable. In previous discussions of the secular acceleration problem the emphasis has always been on the better timed oldest observations available. The most celebrated ancient eclipse was the one observed by Hipparchus in 128 B.C., to which Fotheringham[11] refers as the critical eclipse of Hipparchus. The tacit assumption was that the amplitude of the fluctuation curve could be considered to have remained the same through twenty or more centuries as during the three centuries for which accurate, more or less continuous observations exist. If the amplitude of the fluctuation curve must be considered to increase proportionately to $(-T)^{3/2}$ from the present epoch, the principal uncertainty is that due to the accumulation by the random process rather than the observational uncertainty. Hence the best determination of the secular acceleration in the moon's motion is to be obtained from a collection of reliable observations as well distributed in time as possible rather than from the most ancient and best timed observations.

6. While changes in atmospheric and oceanic circulation appear to be adequate to explain the small seasonal changes in the earth's rotation, this cause must be ruled out as a possible explanation of the cumulative random changes in the length of the year. The mass of the earth's atmosphere is 8.6×10^{-7} times the mass of the earth. If one-half of the atmosphere, between latitudes 30°N and 30°S acquired a drift velocity of 7 miles per hour eastward, the length of the year would be decreased by 0.09 second. In order to account for changes in the length of the year as observed during the past century, drift velocities with a range of about 200 miles per hour would be required. Over longer intervals of time the cumulative effect would require even more prohibitive drift velocities.

It has long been recognized that the cause of the changes in the earth's rate of rotation that produce the fluctuation curve must be sought in changes in the moment of inertia about the axis of rotation. If the slow gradual changes in the length of the day by tidal friction are ignored, the condition is that from year to year

$$C\Omega = \text{constant},$$

C being the moment of inertia about the axis of rotation, Ω the angular ve-

locity of rotation. For a sequence of successive years the average values of C and Ω will be

$$\begin{array}{ll} C_0 & \Omega_0 \\ C_0(1+\epsilon_1) & \Omega_0(1-\epsilon_1) \\ C_0(1+\epsilon_1+\epsilon_2) & \Omega_0(1-\epsilon_1-\epsilon_2) \\ C_0(1+\epsilon_1+\epsilon_2+\epsilon_3) & \Omega_0(1-\epsilon_1-\epsilon_2-\epsilon_3) \end{array}$$

the ϵ's being random increments with mean value zero and a standard deviation 2.9×10^{-9} to account for a random cumulative change with standard deviation 0.09 second in the length of the year. These ϵ's may well be accumulations of smaller random changes with intervals much smaller than one year. The astronomical evidence does not throw further light on this question.

The result of the preceding discussion is, therefore, that the observed irregular changes in the earth's rate of rotation may be the result of minute cumulative random changes in the moment of inertia about the axis of rotation. No large sudden changes of any sort need be postulated. This may, unfortunately, make it difficult to find a connection between geophysical phenomena and the physical cause of the changes in the earth's rate of rotation.

7. The uncertainty in the coefficient of the T^2 term in the moon's mean longitude has been found to be much greater than had been obtained formerly. Owing to the relation between the coefficient of the T^2 term in the sun and that in the moon as found by Spencer Jones from a discussion of the modern observations, this does not bring into serious question whether or not the earth's rotation is being slowed down by tidal friction. The provisional result $s_r = -3''.0 \pm 5''.5$ gives for the T^2 terms due to tidal friction and for prediction of the future:

in the moon's mean longitude, $(+2''.2 \pm 5''.5)T^2$;
in the sun's mean longitude, $(+1''.01 \pm 0''.41)T^2$.

The secular acceleration in the sun's mean longitude being entirely due to the secular decrease of the earth's rate of rotation gives for the change in the length of the day $+0^s.00135 \pm 0^s.00055$ per century.

One of the principal theoretical difficulties in this connection has been that of accounting for the ratio between the coefficients of the T^2 terms in the mean longitudes of sun and moon. Jeffreys[12] gave the theoretical relation

$$\frac{\nu}{\nu_1} = \frac{\frac{\kappa-3}{\kappa}N + N_1}{N + N_1}\,\frac{n}{n_1},$$

$-N$ and $-N_1$ being the retarding couples acting on the earth due to the

lunar and solar tides, respectively, n and n_1 the mean motions and ν and ν_1 the coefficients of the T^2 terms in the mean longitudes of the moon and the sun, respectively. The quantity κ is the ratio between the orbital angular momentum of the earth-moon system and the rotational angular momentum of the earth. The present value of κ is 4.82. The minimum value of the ratio ν/ν_1 is 5.0, if N_1 is negligibly small compared with N. On two different assumptions Jeffreys obtains $N/N_1 = 5.1$ and 3.4, respectively. These give 6.3 and 7.2 for the corresponding values for ν/ν_1. With $+5''.22$ and $+1''.23$ for the coefficients of T^2, $\nu/\nu_1 = 4.24$ is below the theoretical minimum. The provisional values found in this discussion, $+2''.2$ and $+1''.01$, with $\nu/\nu_1 = 2.18$ emphasize the contradiction.

Dr. Harold C. Urey,[13] in his Silliman Lectures given at Yale University in April, 1951, proposed to modify Jeffreys' formula by introducing as an additional unknown the secular changes in the moment of inertia about the axis of rotation. The formula then may be written

$$\frac{\nu}{\nu_1} = \frac{\frac{\kappa - 3}{\kappa} + \frac{N_1}{N} + \frac{\Omega}{N}\frac{dC}{dt}}{1 + \frac{N_1}{N} + \frac{\Omega}{N}\frac{dC}{dt}} \frac{n}{n_1}.$$

If numerical values are introduced including $\nu = +2.22 + \alpha$, $\nu_1 = +1.01 + 0.0748\,\alpha$, the result becomes

$$\frac{\Omega}{N}\frac{dC}{dt} = -\frac{N_1}{N} - 0.255 + 0.055\,\alpha.$$

Now $\alpha = 0 \pm 5.5$, whence

$$\frac{\Omega}{N}\frac{dC}{dt} = -\frac{N_1}{N} - 0.255 \pm 0.30.$$

With $N/N_1 = 5.1$,

$$\frac{\Omega}{N}\frac{dC}{dt} = -0.45 \pm 0.30;$$

with $N/N_1 = 3.4$,

$$\frac{\Omega}{N}\frac{dC}{dt} = -0.55 \pm 0.30.$$

A secular decrease of the moment of inertia about the axis of rotation is therefore indicated. This is in agreement with Urey's views on the origin and constitution of the earth.

Acknowledgments.—The study presented in this paper was undertaken

as a direct consequence of Professor Harold C. Urey's Silliman Lectures at Yale University in April, 1951. Several questions that arose in discussions following these lectures led me to examine the problem of the fluctuations more fully than I had had occasion to do previously.

I am particularly indebted to my associate at the Yale Observatory, Dr. A. J. J. van Woerkom, who assisted me with numerous calculations and who permitted me to make use of some of the results of the experiments with the accumulation of random numbers which we undertook jointly in an effort to find answers to various problems that presented themselves in this investigation.

About 17 years ago, Dr. Theodore E. Sterne, now at Aberdeen Proving Ground but then at the Harvard Observatory, was working on cumulative errors in periods of variable stars. During a visit to the Yale Observatory he brought up the question whether the fluctuations in the earth's rotation might be explained as the accumulation of random errors. As I remember our discussion, we agreed that the evidence strongly favored large sudden changes, and his suggestion was not further examined.

A paper giving details of the derivation of the data on the fluctuation curve is being prepared for publication in the *Astronomical Journal*. A joint paper by Dr. van Woerkom and myself, also to appear in the *Astronomical Journal*, will be devoted to our experiments with artificially constructed fluctuation curves.

Sir Harold Spencer Jones has remarked that the fluctuations in the earth's rotation resemble observed errors in pendulum clocks that were shown to be affected by frequent small erratic changes. In 1949 he wrote:[14] "It may prove however, that the earth itself is rather like a pendulum in its behavior and that its rate of rotation is liable to frequent and small irregular changes, so that we can at present merely observe their integrated effect." This view is supported by the evidence presented in this paper.

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GASTRIC ANION EXCHANGE: ITS RELATION TO THE IMMEDIATE MECHANISM OF HYDROCHLORIC ACID SECRETION

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Study of surviving frog gastric mucosa has demonstrated that chloride is actively transported and that active transport of chloride is the origin of the distinctive potential maintained across the gastric mucosa.[1] Understanding of the chloride transport system was recognizably incomplete, for ion transport implies some form of ion exchange, demanding an unidentified partner ion. In this communication, evidence is set forth which suggests that the partner ion is bicarbonate. The immediate mechanism of hydrochloric acid formation is interpreted in terms of a chloride-bicarbonate exchange. No attempt will be made to review numerous hypotheses advanced to explain hydrochloric acid secretion.

An obstacle to acceptance of bicarbonate as the chloride partner is anticipated. Bicarbonate would be removed by specific combination with a membrane carrier from a solution with an extremely low bicarbonate concentration at the typical pH of gastric acid secretion. It is outside the scope of this paper to assess this objection.

Prior to presenting and discussing bicarbonate flux data, it is appropriate to consider permeability of the gastric mucosa to bicarbonate ion. Because chloride transport is the source of current continuously generated by the mucosa, certain conditions can be stipulated for the partner ion associated with chloride transport. Simple exchange of two ions will not result in net transfer of charge unless the associated ion either is ultimately metabolized or it itself diffuses back across the membrane along the concentration gradient created by its active transport. The former situation is not readily accessible to study. In the latter case, the transporting membrane must be both highly and selectively permeable to the partner ion. Parenthetically, neither H nor OH appear to be compatible with this requirement.

High selective permeability to a given ion would ordinarily be confirmed by demonstrating a concentration potential. Specifically, for the gastric mucosa, one would anticipate that a higher bicarbonate concentration on the nutrient side would lead to augmentation of the spontaneous potential and, conversely, depression when the bicarbonate concentration is higher on the secretory side.

Sensitivity of the gastric mucosal potential to bicarbonate concentrations is experimentally demonstrable. In a typical experiment, summarized in table 1, the mucosa is initially bathed on both sides by a bicar-

bonate-free, oxygen aerated modified Ringer's solution.[1] Introduction of 19 mM HCO_3 replacing 19 mM Cl, into the nutrient solution buffered with 5% CO_2-95% O_2 increases the spontaneous potential. The potential is restored toward normal by adding bicarbonate similarly to the secretory solution. When the nutrient surface is bathed by a bicarbonate-free solution and the secretory surface by a bicarbonate-containing carbon dioxide buffered solution, the spontaneous potential is reversibly depressed. This appears to be a specific response to the bicarbonate ion. In the absence of added bicarbonate, exposing the secretory surface to either 5% CO_2-95% O_2 or to a markedly reduced chloride concentration does not change the potential significantly. As would be expected, changes of mucosal current parallel those of the potential.

While electrical response of the isolated gastric mucosa suggests that asymmetric bicarbonate concentrations are capable of creating a concentration potential, the response is possibly more complicated and may be subject to other interpretations. Nevertheless, in so far as the bicarbonate

TABLE 1

ELECTRICAL RESPONSE TO BICARBONATE

NUTRIENT SOLUTION	SECRETORY SOLUTION	POTENTIAL, mV	CURRENT, MICROAMP.
−	−	19	40
+	−	28	57
+	+	25	50
−	+	12	18
−	−	22	43

− Bicarbonate-free Ringer's, 100% O_2.
+ 19 mM $NaHCO_3$ Ringer's, 5% CO_2-95% O_2.

ion has been studied, the results are consistent with a high degree of selective permeability.

The basis for identifying the partner ion of the chloride transport system as bicarbonate is obtained from a study of bicarbonate–carbon dioxide fluxes, i.e., the separate rates of movement from one side of the mucosa to the other. Apart from increased technical difficulties posed by study of bicarbonate flux, interpretation of bicarbonate flux data is particularly complicated by the bicarbonate–carbon dioxide equilibrium.

The flux data summarized in table 2 were obtained from the surviving frog gastric mucosa. An isolated mucosa was mounted between particular flux chambers which were modified to the extent of each being connected to a separate closed pump circuit recirculating a gas mixture. At the beginning of an experiment, the appropriate gas mixture, the same for the two sides of the mucosa, was bubbled through each system for 15 minutes. Separate pump circuits were then closed and radioactive bicarbonate added to one side to measure a flux rate. Samples for measurement

of C 14 were obtained from the flux chambers through mercury seals. Gaseous dead space was approximately 33 ml./10 ml. of solution. Influx refers to movement from the mucosal to the serosal surface, and outflux to movement in the opposite direction. The chief errors arose from the gaseous dead space, loss of activity from the system (*ca.* 5%/hr.) and back-diffusion.

Both fluxes were measured in two groups of experiments which differed in bicarbonate–carbon dioxide concentrations of the bathing solutions. Within each group, the concentrations were identical on each side of the mucosa and identical for the two fluxes. In one group, the bathing solution was a modified Ringer's solution[1] with 19 mM $NaHCO_3$ replacing 19

TABLE 2

BICARBONATE FLUX

HCO_3, mM		FLUX	FLUX − CURRENT
19.0	Influx	3.8 ± 1.1[a]	$+1.4 \pm 1.1$[a]
19.0	Outflux	1.9 ± 0.7	-0.3 ± 0.6
5.7	Influx	2.1 ± 0.4	-0.8 ± 0.9
5.7	Outflux	0.3 ± 0.2	-2.0 ± 0.4

(microeq./sq. cm./hr.)

[a] Standard deviation.

mM NaCl aerated by 5% CO_2-95% O_2, and for each flux 29 determinations were made on 8 membranes. In the second group, the HCO_3 concentration was 5.7 mM aerated by 1.5% CO_2-98.5% O_2, and for each flux 12 determinations were made upon 3 membranes. In all experiments, the potential across the mucosa was reduced to zero by an external applied potential in series with the mucosa.[2] The electrochemical gradient of bicarbonate across the mucosa was thus zero.

The most striking feature of bicarbonate–carbon dioxide fluxes across the gastric mucosa is the discrepancy between influx and outflux (Table 2). If the electrochemical gradient across a membrane is zero, as in these experiments, influx and outflux of an ion moving solely by passive diffusion will be equal, irrespective of the intrinsic nature of the membrane. The flux asymmetry should not be accepted as evidence of active transport without properly evaluating experimental limitations.

Three experimental limitations deserve comment. First, the flux data represent movement of both carbon dioxide and bicarbonate. However, though measurements are lacking, there is no apparent reason to suspect that there were partial pressure differences of carbon dioxide sufficiently large to account for observed differences of flux. Second, it is possible that incorporation of labeled bicarbonate into the metabolic machinery could give rise to apparent asymmetry. However, this is quantitatively im-

probable in the light of present knowledge of carbon dioxide fixation. Third, asymmetric fluxes might also be encountered when conditions do not favor approach to a steady state, as in the case of potassium fluxes.[3] However, the extracellular/intracellular ratio and high rates of flux favor attainment of a steady state. Thus it seems reasonable to accept the asymmetric fluxes of bicarbonate–carbon dioxide as evidence for active transport of either bicarbonate or carbon dioxide.

The next step in interpreting the role of bicarbonate transfer across the gastric mucosa will be to link the quantitative aspects of bicarbonate–carbon dioxide fluxes to the previously reported chloride transport system.[1] Before clarifying the relationship a digression is necessary. In an abstract situation where a single membrane separates two bulk solutions, quantitative aspects of the fluxes can be simply stated. If such a membrane actively exchanged chloride against bicarbonate with a passive back diffusion of bicarbonate such that a current were generated equivalent to the net transfer of chloride, the outflux of bicarbonate would have to be at least equal to the membrane current. This obviously is not satisfied by the gastric mucosa because outflux may be considerably less than the membrane current, especially in the presence of 5.7 mM HCO_3 (table 2, Column: flux − current).

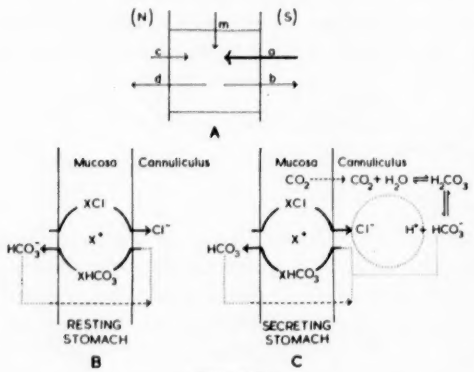

FIGURE 1

So simplified a model is inadequate for the gastric mucosa. In fact, there are at least two membranes in series, constituting the two faces of the cell exposed to the bulk solutions. When dealing with two or more cell membranes in series, it is necessary, as pointed out by Ussing,[4] to resolve the over-all fluxes from one bulk solution to the other bulk solution into individual fluxes across each membrane.

A simplified situation for the gastric mucosa is represented diagrammatically in figure 1 (*A*). Separate fluxes are represented by arrows. Movement of bicarbonate from bulk solution *S* into the cell, flux *a*, is accomplished by active transport (forced chloride-bicarbonate exchange). Generation of an electric current involves passive backdiffusion of bicarbonate, flux *b*. Fluxes *c* and *d* represent passive diffusion in and out of the cell across its opposite cell surface, their relative values determined by the

electrochemical gradient. M is the contribution of metabolic bicarbonate. In the steady state, the sum of the fluxes into the cell will equal those out of the cell, and the over-all flux (outflux) from bulk solution N to bulk solution S will be:

$$c \cdot \frac{b}{b + d}$$

Thus the over-all outflux between the two bulk solutions need only be a fraction of the passive backdiffusion (b) across one membrane required for generation of an electric current and the observed bicarbonate fluxes are compatible with bicarbonate fulfilling the role of the chloride partner ion.

An incidental feature of this model (figure 1 (A)) is that the relative discrepancy between influx and outflux is determined by the extracellular/intracellular bicarbonate concentration ratio. Without presenting algebraic derivation for this assertion, attention can be drawn to the data. Results agree with expectations. Changing the extracellular bicarbonate from 19 mM to 5.7 mM only decreases influx to $^1/_2$ while outflux is decreased to $^1/_6$ (Table 2).

Origin of hydrochloric acid from anion exchange can be most readily understood by considering circumstances that would obtain first in the resting stomach and then in the secreting stomach. Active anion exchange of the resting stomach is depicted in figure 1 (B). Specific affinity of an unidentified membrane carrier X changes at two interfaces so that chloride is actively exchanged against bicarbonate, resulting in a net transfer of chloride to the secretory side. The gastric mucosa is selectively permeable so that bicarbonate quantitatively diffuses back along its concentration gradient resulting from its active transport. There is no net transfer of bicarbonate because it is continuously recirculated. Net transfer of chloride, carrying a charge, generates a continuous mucosal current, usually recognized as the distinctive gastric mucosal potential. In the resting state, with sodium diffusing along the potential gradient there will be a neutral bicarbonate free chloride secretion, in agreement Obrink's findings.[5]

The secreting stomach, depicted in figure 1 (C), differs from the resting stomach by a marked increase in its metabolism and passage of excess carbon dioxide into the canaliculus.[6] Resultant carbonic acid in the canaliculus provides further bicarbonate to displace chloride on the membrane carrier leading to a markedly increased net transfer of chloride. While active transport of bicarbonate from canaliculus to plasma is similarly increased, passive backdiffusion of bicarbonate need not be appreciably greater than in the resting state. Unless membrane permeability changes, backdiffusion will be proportional to fairly similar intracellular and plasma bicarbonate ion concentrations, and the mucosal current will be similar to

that of the resting stomach. The picture is completed by observing that the residual hydrogen ions from intracanalicular carbonic acid, together with actively transported chloride constitute the hydrochloric acid secretion.

In this interpretation no specific transport mechanism is assumed for hydrogen ion as such. No evidence has yet been presented to justify prevalent contentions that there is a specific hydrogen ion transport system in the gastric mucosa. It should be emphasized that chloride-bicarbonate exchange is endergonic. Exergonic metabolic reactions geared to the carrier change its relative affinity at two interfaces.

At present this explanation for the secretion of hydrochloric acid can only be considered tentative. However, it has the advantages of requiring the least number of assumptions for ion transfer across the gastric mucosa. Unequivocal demonstration of active bicarbonate transport will mean that one major ion transport system suffices without postulating separate systems for secretion of chloride and hydrogen. At best, it can only be taken as the immediate mechanism for hydrochloric acid formation. The unidentified membrane carrier and the routes for necessary transfer of energy remain enigmas.

It is a very great pleasure to acknowledge the debt to Professor H. H. Ussing for suggesting this problem and for his continued stimulus.

Summary.—Study of bicarbonate-carbon dioxide flux across the gastric mucosa has demonstrated that their movement is greater from the secretory to nutrient surface than in the opposite direction, contrary to the case for chloride. Active bicarbonate transport satisfactorily explains the flux asymmetry. Quantitative aspects are compatible with a forced exchange of chloride against bicarbonate. Anion exchange is discussed as an explanation for the immediate mechanism of hydrochloric acid formation by the gastric mucosa.

* National Research Council Fellow in Medical Science.

[1] Hogben, C. A. M., PROC. NATL. ACAD. SCI., **37,** 393–395 (1951). In this article, an important proofreading oversight should be noted. Tabulation at end (p. 395): the columns of figures under sodium influx and sodium outflux sould be interchanged. Equation at bottom of p. 393: Vm should of course be mV (millivolts).

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[6] In figure, cannuliculus should read canaliculus.

*A HEMOCHROMOGEN COMPONENT OF LIVER MICROSOMES**

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Submicroscopic particles (microsomes) isolated from the livers of various animals by means of differential centrifugation are obtained in the form of a transparent jelly with a distinct amber color.[1, 2] The nature of the coloring matter of these particles has not been thoroughly investigated. The fact that microsomes possess little oxidative enzyme activity[2] suggests that the amber hue is not due to the normal cytochrome pigments associated with biological oxidations. Bensley[3] has extracted with lipid solvents from microsomes a red colored material which he suggests is lipid in nature. In this communication we wish to present evidence that the major intrinsic pigment of liver microsomes is a hemochromogen compound resembling, but distinct from, the well known cytochromes *a*, *b* and *c* first described by Keilin.[4]

Materials and Methods.—The tissue used in these studies was rat liver, perfused *in situ* with cold isotonic saline to remove blood pigments which might otherwise contaminate the homogenate prepared from it. The perfused liver was homogenized in 9 volumes of ice-cold 0.25 *M* sucrose, and the homogenate fractionated by differential centrifugation at 0–5°C.[5] The microsome fraction so obtained presented a typical transparent, dark amber appearance. Finally, to wash away pigments that might be merely adsorbed, the microsome pellets were rehomogenized in ice-cold isotonic saline and recovered by high-speed centrifugation.

For the spectrophotometric studies, washed microsome pellets were treated with a solution of 1% Na desoxycholate in 0.05 *M* glycylglycine buffer, pH 7.0 in a manner similar to that described by Ball, Strittmatter and Cooper[6] for a heart muscle preparation. The slightly turbid preparation obtained was centrifuged at 110,000 times the force of gravity for 15 minutes at 0–5° and the clear orange fluid removed by pipette from between a minute colorless precipitate and a white scum-like surface layer. These desoxycholate preparations were prepared so that they contained the microsomal material from 0.5 g. of liver in each milliliter of solution.

Samples of this microsome preparation were placed in special vessels consisting of a Thunberg tube fused to a standard Beckman corex cuvette. When atmospheres other than air were required, the vessels were evacuated 3 times, flushed and equilibrated with the desired gas. Substrates, if any, were tipped in from the hollow Thunberg stopper, and absorption measurements made in a Beckman model DU spectrophotometer at room temperature. Readings were made every 1 or 2 mμ in regions of maximal

and minimal absorption, and at 5 mμ intervals elsewhere. The wave-length scale of the instrument was calibrated against various lines of a Hg lamp.

Examinations using a hand spectroscope were made on both desoxycholate preparations and on suspensions of washed microsomes in 0.05 *M* glycylglycine or phosphate buffer at pH 7.0.

The protein (plus nucleoprotein) content of the liver fractions was determined by precipitation with 10% trichloracetic acid. The precipitate was washed once with 50% ethanol and once with 95% ethanol. The lipids were then removed by extracting 3 times with boiling 2:1 ethanol:ether mixture. The residue was dried at 110°C. for 1 hour, cooled *in vacuo* over calcium chloride and weighed.

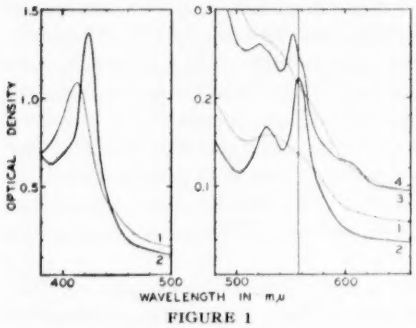

FIGURE 1

Absorption Spectra of Liver Microsomes and Mitochondria. Curves 1 and 2 are observations on microsomes. Curves 3 and 4 are observations on mitochondria. Curves 1 and 3 represent the oxidized state and curves 2 and 4 are obtained after reduction by sodium dithionite under nitrogen. In each case, the liver particles were isolated by differential centrifugation, washed, and homogenized in a 1% solution of sodium desoxycholate in 0.05 *M* glycylglycine buffer, pH 7.0. After clarification by centrifugation, the resulting preparation was used for the spectroscopic observation. In each case 1 ml. contained the material isolated from 0.5 g. of liver. The length of the light path in these observations is 1 cm. and the slit width is 0.06 mμ between 660 and 626 mμ and 0.04 mμ between 625 and 380 mμ.

Results.—The absorption spectra of desoxycholate preparations of microsomes in the oxidized and reduced state are shown by curves 1 and 2 of figure 1. The oxidized form, obtained in air or nitrogen, shows a slight broad hump at 525–535 mμ and a typical Soret band at 414 mμ. When reduced with dithionite ($Na_2S_2O_4$) under nitrogen, there appears a sharp band at 557 mμ and a lesser band with a maximum at 527 mμ, while the Soret band shifts to 424 mμ. The spectra are typical of a hemochromogen. The bands in the visible can readily be seen with a hand spectroscope, both with the desoxycholate preparation and the buffered microsome suspensions. No absorption bands of other materials such as cytochrome *a*, *b* or *c* were noted in the range 700 to 380 mμ, though they may be present in low concentrations and be masked by the intense absorption of this hemochromogen.

To determine the type of heme present in this hemochromogen, microsomes were treated in an alkaline solution with pyridine and dithionite. Absorption bands appeared which were narrower and much more intense

than those seen in reduced microsome preparations. The absorption spectrum of this solution, as determined in the Beckman spectrophotometer, was the same as that described for reduced pyridine protoporphyrin hemochromogen.[7] Using the extinction coefficients as given by Drabkin[7] for the bands of pure pyridine protoporphyrin hemochromogen at 557 mμ and 527 mμ, we have calculated from this absorption spectrum, the heme content of the microsomes. The results are equivalent to 1.45×10^{-6} mols heme per gram microsome protein or 3.75×10^{-8} mols per gram of fresh liver. This is a value about three times that of the concentration of cytochrome *c* in heart muscle (cf. 6).

The hemochromogen content of the microsomes was reasonably constant ($\pm 15\%$) in preparations from a number of rats. Neither the concentration of the hemochromogen nor the postion of its absorption bands was altered by a variety of experimental procedures. In preparations from thoroughly perfused livers, no spectroscopic alterations were caused by washing the microsomes with different solvents (0.05 *M* glycylglycine, pH 7.0; 0.05 *M* phosphate buffer, pH 6.0–8.0; 0.25 or 0.88 *M* sucrose; or water), by washing several times, or not washing at all. Nor was perfusion necessary, provided that an effective wash, either isotonic saline or phosphate buffer, was used to remove the blood pigments which will adhere to microsomes prepared in hypertonic sucrose. In view of these facts and that the techniques and conditions employed in the preparations were such as to produce the least possible damage to the material studied, it seems reasonable to conclude that this hemochromogen is an integral part of the microsomes.

It should also be pointed out that the hemochromogen is concentrated in the microsomes. If it is present in other liver fractions, then its concentration is too low to be distinguished in the spectra we have measured. For examples, the spectra of a washed liver mitochondrial fraction treated with desoxycholate in the same manner as described for the microsomes are shown in figure 1. In the reduced preparation the bands of cytochromes *a*, *b* and *c* are clearly discernible, absorbing at 605, 562 and 551 mμ, respectively, but there is no indication of a compound absorbing preferentially at 557 mμ.

Various compounds were added to microsome solutions under anaerobic conditions to test whether they would reduce the hemochromogen. Addition of a 10^{-3} *M* solution of reduced diphosphopyridine nucleotide produced instantly a 90% reduction as measured by comparing the height of the absorption bands with those obtained by adding dithionite. Addition of ascorbate produced about 60% reduction, while no measurable reduction was observed upon the addition of succinate, lactate, or para-phenylenediamine. Added cytochrome *c* can be reduced in the presence of the microsome preparation upon the addition of reduced diphosphopyridine nu-

cleotide or succinate. A partial reduction of the hemochromogen occurs when the microsome preparation is allowed to remain several hours at room temperature under thoroughly anaerobic conditions in the absence of any added substrate.

Preparations of the hemochromogen that have been reduced by dithionite or reduced diphosphopyridine nucleotide are reoxidized when equilibrated with air. Since reoxidation is not inhibited by 0.1 *M* KCN, which inhibits reoxidation of added reduced cytochrome *c*, and since urethane and SN 5949, an antimalarial naphthoquinone,[8] are without effect in concentrations at which they inhibit oxidative reactions, it is reasonable to assume that the hemochromogen is autoxidizable.

The behavior of the pigment upon the addition of various classical hemochromogen reagents was observed. Addition of 0.01 *M* KCN, 0.1 *M* urethane or 10^{-4} *M* SN 5949 to the oxidized or reduced microsome solution produced no observable spectrum change. Equilibration of a reduced solution with carbon monoxide did not cause a shift of the absorption peaks. When a microsome suspension in the oxidized state was equilibrated with nitric oxide, absorption bands in the visible range appeared at 570 and 535 mμ. These bands are, respectively, 12 mμ and 8 mμ higher than the bands of the reduced form. An identical spread was found between the bands of the nitric oxide compound of cytochrome *c* and its reduced form.

A rough estimate of the oxidation-reduction potential of the hemochromogen was obtained by titrating with dithionite, under nitrogen, a suspension of microsomes in glycylglycine buffer, pH 7.0. An oxidation-reduction indicator dye of suitable potential was added. The degree of reduction of the hemochromogen was observed with a hand spectroscope during the course of the titration; and the potential at each point was estimated by comparing the intensity of dye color in the microsome solution with a series of standard dye solutions. The usual precautions were taken to compensate for the color of the microsome solution. The results of these determinations, given in table 1, indicate that the E_0' value for the hemochromogen at 23°C. and pH 7.0 lies in the neighborhood of −0.12 volt.

Discussion.—It has long been known that liver and a few other mammalian tissues possess an anomalous cytochrome spectrum.[12] Whereas the reduced spectra of homogenates from most actively metabolizing mammalian tissues show distinct bands of cytochromes *b* and *c* at approximately 562 mμ and 550 mμ respectively, similar preparations from whole liver show in this region a broad, poorly defined band, with a maximum near 560 mμ, which is very similar to the so-called cytochrome b_1 found in yeast grown under anaerobic conditions[13] and in other micro-organisms.[14] It has been recorded above that the spectra of liver mitochondria, the particulate fraction which contains most of the oxidative enzyme activity of the cell, show

the presence of the normal cytochrome components. It may therefore be suggested that the anomalous spectrum of liver results from the masking of the normal cytochrome absorption spectrum of the mitochondria by the intense absorption of the microsome hemochromogen.

Recently Yoshikawa[15] has obtained from whole liver, by the method of Keilin and Hartree[16] for preparing cytochrome oxidase, a preparation which on spectroscopic examination shows absorption bands at 560 mμ and 525 mμ. Following the nomenclature of Fujita and Kodama[14] he calls this

TABLE 1

ESTIMATION OF THE OXIDATION-REDUCTION POTENTIAL OF THE MICROSOME HEMOCHROMOGEN SYSTEM (TEMPERATURE = 23°C.; pH = 7.0)

INDICATOR	% REDUCTION OF INDICATOR					REFERENCE
	ca. 5%	25%	50%	75%	*ca.* 95%	
2,6-Dichlorophenol indophenol	− (+0.255)	− (+0.231)	− (+0.217)	− (+0.203)	− (+0.179)	9
Toluylene blue	− (+0.153)	− (+0.129)	− (+0.115)	− (+0.101)	− (+0.077)	9
Methylene blue	− (+0.039)	− (+0.025)	− (+0.011)	− (−0.003)	− (−0.027)	9
Indigo trisulfonate	− (−0.043)	− (−0.067)	− (−0.081)	+ (−0.095)	++ (−0.119)	9
Indigo disulfonate	− (−0.087)	+ (−0.111)	++ (−0.125)	+++ (−0.139)	+++ (−0.163)	9
Nile blue	+ (−0.089)	+++ (−0.113)	+++ (−0.127)	+++ (−0.141)	+++ (−0.165)	10
Indigo monosulfonate	+ (−0.118)	+++ (−0.142)	+++ (−0.156)	+++ (−0.170)	+++ (−0.194)	9
Janus green	+++ (−0.218)	+++ (−0.242)	+++ (−0.256)	+++ (−0.270)	+++ (−0.296)	11

Numbers in parentheses indicate the potential at that point in the titration, as calculated from the E_0' values of the dyes at 30°C. as given in the reference cited.

+ and − signs indicate state of reduction of the hemochromogen: − = oxidized; + = 25% reduction; ++ = 50% reduction; +++ = >95% reduction.

hemochromogen cytochrome b_1. The bands of this component were also observed in kidney and ovary though not in heart or brain tissue. This hemochromogen is autoxidizable and it forms an oxidation-reduction system with a potential estimated as −0.13 volt at pH 7.5 and 15°C. It would appear to us that the hemochromogen observed by Yoshikawa is identical with the one that we have observed in the microsomes isolated from liver.

Kun[17] has treated washed rat liver particles (presumably mitochondria, microsomes and nuclei) with isobutanol in the presence of carboxymethyl cellulose. He obtained an extract which, on reduction, shows absorption

bands at 560 mμ and 530 mμ. This extract catalyzes the transfer of electrons from reduced DPN or TPN to cytochrome *c*. Kun suggests the possible identity of this component with cytochrome *b*. We believe that his component may be related to the microsome pigment. However, in view of the extraction procedure he uses, no definite conclusion can be made at this time.

The biological function of the microsome hemochromogen is not known to us. The known functions of the microsomes themselves are few: they are known to be active in the incorporation of amino acids into proteins[18] and to have the ability to transfer electrons from reduced DPN to an added acceptor such as cytochrome *c*.[19] The microsomal hemochromogen may well function in the transport of electrons from reduced DPN. If it does, then it seems doubtful that cytochrome *c* can be the acceptor of electrons, since the microsomes contain little, if any, cytochrome *c*. Further, since the microsomes are relatively inactive in carrying out oxidative processes,[2] the microsome hemochromogen does not appear to function in oxidative reactions in place of the normal cytochrome system.

It may be that the hemochromogen is involved instead in reductive synthetic reactions, e.g., the formation of saturated fatty acids and the steroids, wherein a —CH=CH— bond becomes reduced to a —CH_2—CH_2—bond. This hemochromogen forms an oxidation-reduction system with a potential lower than any other known naturally occurring hemochromogen and, indeed, some 100 millivolts negative to the succinate-fumarate system at pH 7.0. It would therefore be a satisfactory agent for the transfer of electrons in this type of reductive synthesis. Its localization in the microsomes and its especially high concentration in the liver would be in keeping with such a role. The liver, with its predominantly venous blood supply, is an ideal organ in which to carry on reductive processes and the microsomes, with their low oxidative capacity, would be a logical site within the liver cell for such processes to occur. Thus the mitochondria could be looked upon as the site of oxidative reactions supplying high energy phosphate bonds and the microsomes as the site of reductive synthetic processes. The reduced pyridine nucleotides would supply the grist for both these mills.

Summary.—The major intrinsic pigment of liver microsomes is a protoporphyrin hemochromogen that resembles but is distinct from the cytochromes, *a*, *b* and *c*, which are not spectroscopically detectable in the microsomes. This hemochromogen forms an oxidation-reduction system with an E_0' of approximately −0.12 volt at pH 7.0. The oxidized form shows a sharp band at 414 mμ and a broad band at 525–535 mμ which is altered in the presence of nitric oxide but not cyanide. The hemochromogen is reversibly reduced by dithionite, reduced diphosphopyridine nucleotide, or ascorbate, with the appearance of bands at 557, 527 and 424

mμ. These bands of the reduced form are unaffected by CO. The concentration of the hemochromogen is estimated to be equivalent to 3.75×10^{-8} mol of hemin per gram of wet liver, a value three times that for cytochrome *c* in heart muscle. It is concluded that this pigment, concentrated only in the microsomes, is probably the previously described "cytochrome-b_1" component responsible for the anomalous cytochrome spectrum of liver and certain other tissues.

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*INCOMPLETE GENETIC BLOCKS IN BIOCHEMICAL MUTANTS OF NEUROSPORA**

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Introduction.—Mutant strains of micro-organisms, requiring for growth the addition of a specific vitamin, amino acid or similar vital substance, might reasonably be expected to be incapable of performing particular enzyme-catalyzed reactions. Such an expectation seems to be supported

by the observations that these mutants require specific supplements for initiation of growth, and that the growth response to these compounds is a function of the amount supplied, i.e., growth curves are of the type expected if growth were entirely dependent on an exogenous supply of a specific substance. The fact that only those strains showing no growth, or virtually no growth, on minimal medium are customarily saved for study would also lend credence to the thesis that these strains have completely lost a particular biosynthetic ability. However, in our use of such mutants for biochemical and genetic studies the question arose to what extent is one justified in assuming that these strains cannot synthesize the particular compounds they require for growth.

In the course of investigations of niacin and tryptophan formation in Neurospora, evidence has been obtained suggesting that growth behavior may not provide entirely reliable criteria for the purpose of judging whether a strain can or cannot form the compounds required by it for growth. Accordingly more extensive studies have been carried out with a variety of mutant strains to determine whether they are capable of synthesizing the compounds they require for growth. These tests, in general, have permitted the examination of the ability of mutant strains to form the required compounds under growing conditions. Two general experimental methods have been employed in this work: the use of tracer atoms and the measurement of the accumulation of specific intermediates by double mutants.

If genetic alterations do lead to a complete loss of ability to perform particular biochemical reactions, tracer atoms, incorporated into intermediates after the points of block, would be expected to be found in equal concentration in the end products. Thus, the completeness of a genetic block can be judged by the dilution of tracer in an end product, provided no exchange of tracer occurs and no alternative paths of end product formation are functional.

The completeness of genetic blocks may also be examined experimentally by a second and different technique. Many mutant strains of Neurospora are known to accumulate characteristic compounds. The accumulation of such compounds by double mutants may be used in judging the completeness of a genetic block. The basis of the use of double mutants can be most easily shown diagrammatically:

$$C \xrightarrow{\text{gene 3}} B \xrightarrow{\text{gene 2}} A \xrightarrow{\text{gene 1}} EP$$

Assume that a given end product (EP) is formed through a sequence of reactions involving three other intermediate compounds. Gene 1 controls the conversion of A to EP and, if defective, causes the accumulation of A. Gene 2 controls the conversion of B to A. The double mutant having

defects in both gene 1 and gene 2 should not accumulate *A*, provided the alteration of gene 2 has resulted in a complete block of the ability to convert *B* to *A*. Gene 3 is concerned with the conversion of *C* to *B*. The double mutant having defects in genes 1 and 3 likewise should not accumulate *A* if grown in the absence of *B*. However, if *B* is present, *A* should be accumulated and, in turn, the extent of accumulation of A should be a function of the amount of *B* added. This method is valid, of course, only if there are no alternative methods of formation of *A*.

Mutants requiring niacin for growth and mutants requiring tryptophan for growth were used in this work. These mutants are particularly suitable since it has been shown that niacin is formed in Neurospora solely through a series of reactions involving tryptophan as the initial specific precursor.[1, 2] Thus, alternative pathways of niacin formation in Neurospora do not occur. It has further been shown that the ring nitrogen of tryptophan is not exchanged during niacin formation;[2, 3] thus exchange is not a problem if the tracer employed is N^{15}. Lastly, a majority of the compounds intermediate between tryptophan and niacin are known.[4, 5]

Our knowledge of tryptophan formation is not as satisfactory as is our knowledge of niacin synthesis. Tryptophan is formed by the condensation of indole and serine[6] and indole, in turn, is formed from anthranilic acid.[2, 7] Whether indole is formed solely from anthranilic acid cannot be definitely stated. However, since strains requiring tryptophan, and differing from the parental strain by a single gene in each case, are readily found, it seems probable that only one major pathway of tryptophan formation is functional in Neurospora. Thus the pathway of tryptophan and niacin formation represents a system well characterized biochemically, for which a number of genetic blocks are known.

The Evidence for Incomplete Genetic Blocks.—Attention was drawn to the possibility of partial genetic blocks through studies of strain 39401. This strain differs from the parental strain in a single gene,[8] yet it will grow if furnished either tryptophan or niacin.[1] Since this tryptophanless strain can also use indole but not anthranilic acid for growth, it is presumed to be blocked in the conversion of anthranilic acid to indole. It is surprising, therefore, that strain 39401 will grow on niacin alone, as niacin is formed solely from tryptophan in Neurospora. The genetic alteration characteristic of this strain must then be such as to permit tryptophan formation when this strain is grown on niacin. This has been verified in a tracer experiment using N^{15}-labeled anthranilic acid[2] in which strain 39401 was found to convert anthranilic acid to tryptophan under growing conditions. Thus, in this instance, gene change has altered the ability of a strain to form tryptophan, yet has not rendered it incapable of forming tryptophan under all conditions.

To test the uniqueness of the behavior of strain 39401, four additional

tryptophanless strains were studied [strains C-83,[9]† 10575,[7] 75001[7] and 7655[10]]. Each strain was genetically combined with strain 3416 to form the double mutants. Strain 3416 requires niacin for growth and accumulates generous quantities of quinolinic acid (pyridine-2,3-dicarboxylic acid) in its culture medium.[11] The procedural details of these experiments, including the methods of culture on N^{15}-labeled compounds as well as the isolation and characterization of quinolinic acid and tryptophan, have been given in a previous publication.[2]

In one experiment double mutant C-83–3416 was cultured on N^{15}-labeled tryptophan and unlabeled niacin. The tryptophan of the mycelium formed and the quinolinic acid present in the culture medium were isolated and assayed for N^{15}. As may be seen in table 1, the isolated tryptophan and quinolinic acid both reflect the same N^{15} content as the fed tryptophan. Thus the presence of the mutant C-83 gene in this strain results in a complete inability to form tryptophan.

TABLE 1

N^{15} STUDIES

STRAIN	LABELED SUBSTRATE	MG./18 LITERS	CULTURE PERIOD, DAYS	COMPOUND ISOLATED	N^{15} CONTENT % OF THEORY
C-83-3416	DL-trypto-phan	1433	7	N-acetyl tryptophan	98.9
				quinolinic acid	94.3
10575-3416	Indole	200	7	N-acetyl tryptophan	80
				quinolinic acid	75
75001-3416	Anthranilic acid	250	4	Tryptophan	77
				quinolinic acid	103
75001-3416	Anthranilic acid	80	4	Tryptophan	20
				quinolinic acid	52
75001-3416	Anthranilic acid	80	8	Tryptophan	16.4
				quinolinic acid	29
7655-3416	Anthranilic acid	300	7	N-acetyl tryptophan	37
				quinolinic acid	40

Strain 10575 similarly requires tryptophan for growth, but indole can satisfy its tryptophan requirement. An experiment was carried out with double mutant 10575-3416, similar to that just described for strain C-83-3416, except that N^{15}-labeled indole was used for growth in place of tryptophan. The results of this experiment show, in distinction to the results of the C-83–3416 experiment, that only 75–80% of the tryptophan and quinolinic acid synthesized was derived from the indole fed. Twenty to twenty-five per cent of this tryptophan and quinolinic acid was unlabeled and must therefore have been derived from the constituents of minimal medium. These results indicate that the 10575 mutant gene does not completely prevent indole formation.

Tryptophanless strains 75001 and 7655 can utilize either indole or anthranilic acid in place of tryptophan. These two strains are genetically different

from each other and presumably are blocked in different steps in anthranilic acid formation. Again, double mutants were formed between these mutants and strain 3416. These double mutants were grown on N^{15}-labeled anthranilic acid and the tryptophan and quinolinic acid formed were isolated. From table 1 it can be seen that a substantial portion of the isolated tryptophan and quinolinic acid was derived from sources other than the anthranilic acid fed. These mutants, then, despite their requirement for tryptophan, indole or anthranilic acid, appear to be capable of independent synthesis of these compounds under growing conditions.

These results are consistent with what would be expected if the mutant loci reverted during growth. As a test of this possibility, a generous sample of mycelium was removed from each culture flask at the end of the growth period and placed on an unsupplemented medium and on a medium supplemented with tryptophan and niacin. The conidia formed on supplemented medium were tested for tryptophan independence and for niacin independence. In none of the experiments reported was there any evidence of reversion at any mutant locus.

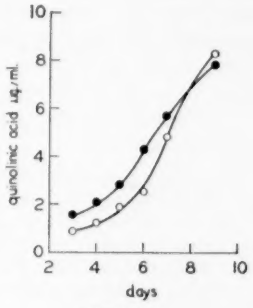


FIGURE 1

Quinolinic acid accumulation by double mutant 10575-3416 grown on two levels of indole. ●——● 12.5 µg./ml./indole, O——O 2.5 µg./ml./indole.

These results are also consistent with what would be expected if exchange of N^{15} occurred. It has been shown that in the conversion of tryptophan to niacin no N^{15} exchange occurs.[2, 3] Exchange of N^{15} in the conversion of anthranilic acid to tryptophan, however, though improbable, has not been similarly ruled out. Therefore, as an independent test of whether or not the strains studied are capable of forming tryptophan *de novo*, a different series of experiments was carried out. As mentioned earlier, niacin is formed solely from tryptophan in Neurospora. The accumulation of quinolinic acid, then, by a double mutant of a tryptophanless strain and strain 3416, should reflect the amount of tryptophan or utilizable tryptophan precursor supplied. Accordingly strain 10575-3416 was grown on two levels of indole and the quinolinic acid present in the culture filtrate measured over a 9-day growth period. As may be seen from figure 1, quinolinic acid accumulation is not proportional to indole concentration; in fact, on the low level of indole, the amount of quinolinic acid formed was greater than that which would have resulted from the quantitative conversion of the added indole to quinolinic acid. A similar experiment was carried out using strain 75001-3416, and is summarized in table 2. Here, too, quinolinic acid accumulation failed to vary in proportion to the amount

of tryptophan precursor supplied. Similar experiments with other double mutants involving indole accumulation also demonstrate the incomplete nature of the genetic blocks characteristic of strains 10575 and 75001. By the introduction of a suitable secondary block, these strains were shown to be capable of forming indole, a compound beyond the point of block. Thus, these experiments confirm the earlier conclusion that certain tryptophanless strains, despite their inability to grow in the absence of tryptophan or a utilizable tryptophan precursor, are nevertheless capable of forming tryptophan under growing conditions.

As stated earlier, the possibility of indole synthesis via secondary biochemical pathways has not been ruled out. Thus, the tryptophan synthesis observed under growing conditions might be a reflection of the operation of such an alternative pathway. Since it has been conclusively shown that niacin is formed solely from tryptophan in Neurospora even under growing conditions it seemed desirable, from the standpoint of a fuller

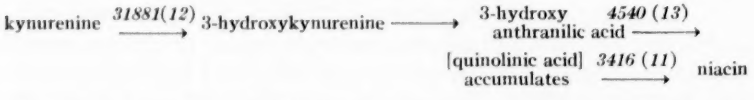
TABLE 2

QUINOLINIC ACID ACCUMULATION BY DOUBLE MUTANT 75001-3416[a]

SUPPLEMENT	QUINOLINIC ACID, FOUND, μG	YIELD FROM ANTHRANILIC ACID, %
40 μg. niacinamide, 50 μg. anthranilic acid	177	290
40 μg. niacinamide, 100 μg. anthranilic acid	188	154
40 μg. niacinamide, 200 μg. anthranilic acid	177	73
40 μg. niacinamide, 400 μg. anthranilic acid	213	44
40 μg. niacinamide, 600 μg. anthranilic acid	236	32
40 μg. niacinamide, 1 mg. anthranilic acid	212	17

[a] 40 ml./flask, shaken 7 days at 25°.

understanding of the mechanism involved, to determine whether niacinless mutants similarly show evidence of the formation of a required substance during growth. Three known genetic blocks in niacin formation may be represented diagrammatically as follows:



Following the reasoning outlined for accumulation studies, double mutants of strain 31881 or 4540 with strain 3416 should not be capable of quinolinic acid formation when grown on a medium supplemented with niacin alone unless either the 31881 or the 4540 block is incomplete. The required double mutants 31881-3416 and 4540-3416 were formed, grown on niacin, and tested for quinolinic acid accumulation. As may be seen from table 3, quinolinic acid was not detected in the filtrate of double mutant 4540-3416. Double mutant 31881-3416, however, did form quinolinic acid. It

must be concluded, therefore, that after growth has been initiated the 31881 block only partially prevents normal niacin synthesis.

Since some of the mutant strains which cannot grow in the absence of tryptophan or niacin are capable of forming these compounds, their ability to do so might reasonably be expected to be reflected in growth. If, under growing conditions, the compound required for growth can be formed, mutants having this ability, when cultured on limiting amounts of required compound, should eventually approach maximum growth. Growth experiments on various levels of tryptophan were therefore performed with strains 10575 and C-83 and the results obtained are compared in figure 2. Strain C-83, as shown earlier, is incapable of tryptophan formation, while strain 10575 can form tryptophan. In the growth experiments strain C-83 was found to reach different final levels of growth, depending upon the concentration of tryptophan supplied. Strain 10575, however, reached about the same level of growth, regardless of the concentration of tryptophan given. At 3 to 4 days, the period usually used for growth measurements, strain 10575 shows a satisfactory growth curve,

TABLE 3

QUINOLINIC ACID ACCUMULATION BY VARIOUS NIACINLESS STRAINS

STRAIN	QUINOLINIC ACID PER FLASK, μG. 3 DAYS	5 DAYS	7 DAYS
31881	0	0	0
31881-3416	0	0	32
4540	0	0	0
4540-3416	0	0	0

with growth a function of tryptophan concentration, while at eight days, growth was equal at all tryptophan concentrations.

Thus, the results from tracer experiments, intermediate accumulation experiments and growth studies are all in agreement with the view that a mutant strain, despite its inability to grow in the absence of a given substance, may nevertheless be capable of forming this substance under growing conditions. This phenomenon will be referred to as "leakage."

Discussion.—Many mechanisms can be postulated to account for leakage. Reversion of mutant loci certainly would account for the data obtained. No evidence of reversion, however, was found. This does not prove that no tryptophan or niacin independent nuclei were present in the grown mycelium, but it strongly suggests that if such niacin or tryptophan independent nuclei were present they were few in number and could not reasonably account for the extensive tryptophan and niacin synthesis observed.

Alternative biosynthetic mechanisms would also account for leakage. The fact that mutants requiring tryptophan can be obtained suggests that

alternative pathways of tryptophan synthesis do not ordinarily contribute significantly to tryptophan formation. This fact is not sufficient, however, to rule out alternative pathways as a cause of leakage. Although alternative pathways might not provide sufficient end product to initiate growth, under growing conditions they might well contribute some end product. Thus, the fact that a mutant requiring niacin does show leakage is of major importance since it has been experimentally demonstrated that niacin is formed solely from tryptophan, even under growing conditions.[2] Alternative pathways of end-product formation will not, therefore, account for all observed cases of leakage.

As neither reversion nor alternative biosynthetic pathways will fully account for all observed cases of leakage, it must be concluded that in certain strains the reaction presumed to be blocked is functional at some period during growth. This is hardly surprising since several instances are now known in which the presence of the enzyme catalyzing the reaction presumably blocked has been demonstrated. Wagner[14] has shown that certain pantothenicless strains of Neurospora possess the enzyme which couples pantoyl lactone and beta-alanine, the presumably blocked reaction characteristic of these mutant strains. The presence of lactase in strains of Neurospora and *E. coli* which are unable to utilize lactose as a carbon source has also been observed.[15, 16]

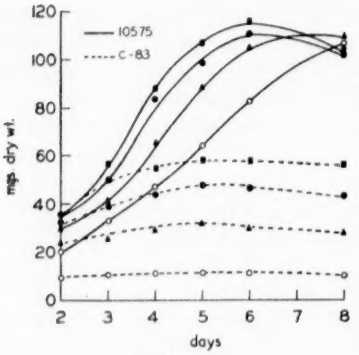


FIGURE 2

Comparison of the growth response of mutants 10575 and C-83 to various levels of tryptophan. ■ = 1 mg. L-tryptophan; ● = 750 μg. L-tryptophan; ▲ = 500 μg. L-tryptophan; ○ = 250 μg. L-tryptophan.

The investigations reported here further show that such incomplete biochemical blocks may be common. Seven out of nine mutant loci examined in Neurospora show leakage, which finding suggests that a large percentage of the biochemical mutant strains of Neurospora may behave similarly. In fact, apparently similar incomplete genetic blocks have been noted by other investigators in Neurospora,[17, 23, 24] [see also review 5], as well as in yeast.[18]

The fact that a reaction presumed to be blocked may be functional under certain conditions implies that genetic change may result in an alteration of a given enzymatic activity. This effect could be brought about by a variety of mechanisms. Alteration of the specificity of an enzyme could lead to a decrease in the rate of the reaction it catalyzes. The rate at which an enzyme is formed could be altered. The time at which an en-

zyme is formed also could be altered. The effect of gene change could be indirect, as for example, through inhibition of enzyme activity; relief from such inhibition at some stage of growth would restore enzyme activity. Evidence exists suggesting that each of these alterations of enzymatic activity can occur as the result of gene change.[19-22] Any one of these changes would lead to a loss in activity of a particular enzyme under certain conditions, although under other conditions the enzyme would be present and functional. Thus any one of these changes might well result in leakage.

In all of the strains studied leakage was found to be more pronounced when low levels of growth supplement were used, and to increase in extent with time. These characteristics might well be expected for any of the possible mechanisms of leakage discussed above. It is not possible, therefore, to give the actual mechanism of leakage for any one mutant.

Thus, while the precise mechanism of leakage is not known for each case studied, the fact that strains may act in this manner is of importance in considering the biochemical and genetic interpretation accorded the behavior of mutant strains. The fact that leakage occurs suggests that a compound might well be found which affects the rate of leakage without directly contributing to the formation of end product. Thus, the observation that a given compound supports the growth of a mutant strain does not of necessity mean that this compound is directly related biosynthetically to the end product required for growth. From a genetic standpoint, these studies suggest that partial biochemical blocks are of common occurrence in mutant strains of Neurospora. It is of interest to note that strains showing leakage were derived from conidia treated with a variety of mutagenic agents; viz., x-rays (10575) ultra-violet light (39401), neutrons (75001) and methyl-*bis* (β-chloroethyl) amine (31881). Thus, gene change does not invariably, and perhaps not even frequently, lead to total loss of enzyme activity. It appears, rather, that a variety of effects on a given enzyme system results from genic change, and that all of these effects must be considered in any general formulation of the nature of the genic control of biochemical reactions.

Summary.—Through the use of tracer atoms and measurement of the accumulation of specific substances, mutant strains of Neurospora, genetically blocked in the formation of niacin and requiring this substance for growth, as well as similar tryptophanless strains, were found capable of synthesizing the specific compounds respectively required for growth. This ability is termed leakage.

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† A culture of this strain was generously supplied by Dr. H. K. Mitchell, California Institute of Technology.

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*THE EFFECTS OF CARBON MONOXIDE AND OXYGEN ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOME ABERRATIONS IN TRADESCANTIA**

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Though the end results of shortwave radiation have been frequently studied and are well understood, little is known concerning the mechanism whereby radiant energy produces its effects. Nor is there specific information concerning the way in which the physiological state of the cell conditions its response to irradiation. The recent reviews of Catcheside,[1] Hayden and Smith,[2] Sax,[3] Sparrow,[4] and Sparrow and Rubin[5] discuss various agents which modify the genetic and cytological effects of x-rays. Among these agents oxygen appears of signal interest.

Experiments of Thoday and Read,[6] Hayden and Smith,[2] Giles and Riley,[7] Giles, Beatty and Riley,[8] Riley, Beatty and Giles,[9] and Conger and Fair-

child[10] have shown that the availability of oxygen to cells is an important factor in radiosensitivity. Under anaerobic conditions radiation-induced inhibition of growth in barley and increase in frequency of chromosomal aberrations (microspores of Tradescantia and root tips of *Vicia faba*) were much less pronounced than in air. Contrariwise, oxygen tensions above the normal 21% caused an increase in chromosomal aberrations in the microspores of Tradescantia—a result which suggests that respiratory metabolism in some way is correlated with the frequency of chromosome aberrations. In the present study this possibility has been examined by studying the relation of heavy-metal-containing respiratory enzymes to the response of Tradescantia microspores to irradiation. The possible role of flavoproteins is also appraised.

Materials and Methods.—*Tradescantia paludosa* Anderson and Woodson was used in all experiments. Cytological observations were made of chromosomal aberration types four to five days after irradiation. The principal types of aberrations analyzed at this period are "interchanges" (dicentrics and centric rings).

The experimental material was placed inside a cylindrical lucite chamber, $4^1/_2$ in. O.D. × 4 in. I.D. × 18 in. long, fitted with brass endplates, gaskets and needle valves. The inflorescences were inserted in an upright position through holes in a waxed cardboard container filled with spring water. The container was placed in the center of the lucite chamber to insure uniform exposure and minimize secondary radiation from the brass endplates. Dosage was measured with a dosimeter placed at the position of the inflorescences in a lucite chamber identical with the experimental chamber. This chamber was placed next to the experimental chamber. In all experiments the dosage rate and total dosage were constant, i.e., 40 r/min. for 10 minutes.

The gases used in the experiments were obtained in commercial cylinders and assayed as follows:

Nitrogen (Airco) 99.5% N_2 plus 0.5% O_2.

Oxygen (Airco) 99.5% O_2 plus 0.5% N_2.

CO (Matheson Co.) 96.8% CO, 0.36% CO_2, 0.97% H_2, 1% N_2, 0.8% saturated hydrocarbons, 1.19 mg. Fe/liter, 0.32 mg. S/liter.

Prior to its use the carbon monoxide was filtered through 10% NaOH solution to remove the CO_2. Total gas pressure was measured by gauges calibrated in psi.

The inflorescences were enclosed in the air-filled lucite chamber and then compressed to the desired pressure with the gas under consideration. When sealed, the chamber thus contained one atmosphere of air plus a positive pressure of the experimental gas. The use of positive pressures permits several peculiar advantages. First, there is no question as to whether the experimental gas reaches the microspores through the layer of

air enclosed by the sepals and petals of the buds. Moreover, since at least 20.9% of an atmosphere of oxygen is present (the amount present normally in air), anoxia can play no role. The average time of compression was four minutes and the timing of exposure to the gas was calculated from the moment the final pressure was attained. The sealed chamber containing inflorescences was kept at room temperature for from 15 minutes to four hours, depending on the experiment, and then placed in the x-ray machine alongside the chamber containing the dosimeter. Irradiation was then performed at room temperature. In each experiment this

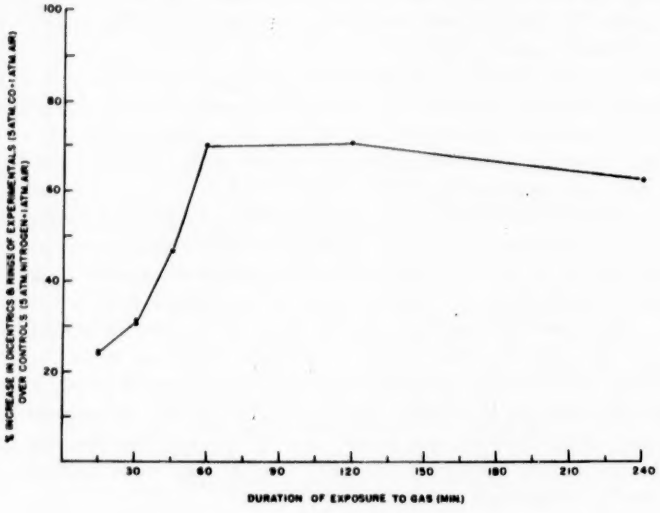

FIGURE 1

Curve showing the effect of time of exposure to carbon monoxide and to nitrogen on dicentrics and centric rings induced in Tradescantia microspores by x-rays.

procedure was carried out twice—once with the experimental gas (usually CO) and once with the control gas (usually N_2). The two experimental chambers were compressed and later irradiated within 30 minutes of each other. After exposure the chambers were decompressed slowly for 30 minutes and then opened to air.

In general, from 10 to 15 inflorescences were exposed in each chamber and as many slides as possible made four to five days following irradiation.

Results and Discussion.—The experimental results are presented in table 1 and shown graphically in figure 1. All the scoring was done by one investigator (E. K.). The first column in table 1 indicates the composition

TABLE 1

FREQUENCIES OF DICENTRICS AND CENTRIC RINGS INDUCED IN TRADESCANTIA MICROSPORES EXPOSED TO X-RAYS IN MIXTURES OF AIR, NITROGEN, OXYGEN AND CARBON MONOXIDE. ALL DOSAGES ADMINISTERED AT A CONSTANT INTENSITY OF 40 R/MIN. FOR 10 MINUTES

(For further discussion, see text)

EXPERIMENTAL				CONTROL			
GAS MIXTURE	DURATION OF EXPOSURE TO gas, min.	CHROMOSOMES COUNTED	PER CENT DICENTRICS AND RINGS	GAS MIXTURE	CHROMOSOMES COUNTED	PER CENT DICENTRICS AND RINGS	PER CENT INCREASE OF DICENTRICS AND RINGS OF EXPERIMENTALS OVER CONTROLS
5 atm. CO + 1 atm. air[a]	60	3000	0.0	5 atm. N_2 + 1 atm. air[a]	3000	0.0	0.0
5 atm. CO + 1 atm. air	60	4200	9.2	5 atm. N_2 + 1 atm. air	3000	9.1	Negligible
5 atm. CO + 1 atm. air	15	1542	11.3	5 atm. N_2 + 1 atm. air	1002	9.0	24.4
5 atm. CO + 1 atm. air	15	1794	10.4	5 atm. N_2 + 1 atm. air	3600	8.4	23.8
5 atm. CO + 1 atm. air	30	3000	12.3	5 atm. N_2 + 1 atm. air	1800	9.4	30.9
5 atm. CO + 1 atm. air	30	600	11.8	5 atm. N_2 + 1 atm. air	1002	9.0	31.0
5 atm. CO + 1 atm. air	45	2712	13.9	5 atm. N_2 + 1 atm. air	3000	9.5	46.3
5 atm. CO + 1 atm. air	60	600	15.3	5 atm. N_2 + 1 atm. air	1002	9.0	70.0
5 atm. CO + 1 atm. air	120	2196	16.1	5 atm. N_2 + 1 atm. air	1200	9.5	70.0
5 atm. CO + 1 atm. air	240	2232	14.8	5 atm. N_2 + 1 atm. air	1800	9.2	60.9
2 atm. CO + 2 atm. O_2 + 1 atm. air	60	2742	10.3	2 atm. CO + 2 atm. N_2 + 1 atm. air	3600	13.9	−25.9
2 atm. N_2 + 2 atm. O_2 + 1 atm. air	60	3672	10.4	4 atm. N_2 + 1 atm. air[b]			16.9
4 atm. O_2 + 1 atm. air	60	3000	11.3	4 atm. N_2 + 1 atm. air	3000	9.0	25.6

[a] This preparation was not irradiated.

[b] This preparation was lost. The per cent increase is calculated on the basis of the value below, since the gas composition of the chambers was the same in both cases.

and pressure of the experimental gas mixture superimposed upon the one atmosphere of air initially present in the chamber. The second column states the duration of exposure prior to irradiation. The third column tabulates the number of chromosomes counted; the fourth indicates the number of dicentrics and centric rings in the experimental preparations. The fifth column records the composition of the gas superimposed on the air in the control chamber. In the sixth and seventh columns the number of chromosomes counted and the per cent dicentrics and centric rings found in the control chamber are listed. In the eighth column is tabulated the per cent increase in dicentrics and centric rings of the experimental preparation when compared with the control preparation. In all experiments except the first one listed, the exposure was 40 r/min. for 10 minutes. In the first experiment tabulated, inflorescences were not irradiated. These results may be summarized as follows:

TABLE 2

FREQUENCIES OF DICENTRICS AND CENTRIC RINGS INDUCED IN TRADESCANTIA MICROSPORES COMPRESSED WITH MIXTURES OF AIR, NITROGEN AND CARBON MONOXIDE EVACUATED, EXPOSED TO AIR AND THEN X-RAYED IN AIR. ALL DOSAGES ADMINISTERED AT A CONSTANT INTENSITY OF 40 R/MIN. FOR 10 MINUTES

(For further discussion, see text)

EXPERIMENTAL				CONTROL			
GAS MIXTURE	DURATION OF EXPOSURE TO GAS, MIN.	CHROMOSOMES COUNTED	PER CENT DICENTRICS AND RINGS	GAS MIXTURE	CHROMOSOMES COUNTED	PER CENT DICENTRICS AND RINGS	PER CENT INCREASE OF DICENTRICS AND RINGS OF EXPERIMENTALS OVER CONTROLS
5 atm. CO + 1 atm. air	60	3000	9.0	5 atm. N_2 + 1 atm. air	3000	9.0	0

1. Irradiation in the presence of positive pressures up to five atmospheres of an indifferent gas such as nitrogen superimposed on one atmosphere of air does not affect the frequency of chromosomal aberrations.

2. Neither high pressures of CO nor high pressures of N_2 cause chromosomal aberrations in the absence of x-radiation.

3. Exposure to CO during irradiation increases the aberration frequency.

4. This increase in aberration frequency depends on the time of exposure to CO prior to irradiation—the longer the exposure time the greater the increase in aberration frequency, with a maximum reached after one hour of prior exposure (figure 1).

5. The CO-induced increase in aberration frequency is almost completely prevented when O_2 is present simultaneously in high concentration.‡

6. Compression with oxygen causes a slight though significant increase in aberration frequency.

Table 2 shows the results of pretreatment with CO on the frequency of x-ray induced aberrations. In this experiment the inflorescences were compressed with five atmospheres of CO for 60 minutes. The chamber was then successively evacuated and filled with air three times to remove the CO. The material was then irradiated as in the previous experiments, the chamber now being filled only with one atmosphere of air. The results of this experiment suggest that:

7. CO must be present *during* irradiation in order for it to have an effect on aberration frequency.

In view of the magnitude of the observed effects, the data presented strongly suggest that CO, when applied simultaneously with irradiation, has a pronounced influence in increasing the radiosensitivity of this material, as measured by chromosomal aberration frequency.

These effects of CO may be analyzed on two different levels. On the

TABLE 3

FREQUENCIES OF DICENTRICS AND CENTRIC RINGS INDUCED IN TRADESCANTIA MICROSPORES EXPOSED TO X-RAYS IN AIR AND THEN COMPRESSED WITHIN 30 SECONDS WITH MIXTURES OF AIR, NITROGEN AND CARBON MONOXIDE. ALL DOSAGES ADMINISTERED AT A CONSTANT INTENSITY OF 40 R/MIN. FOR 10 MINUTES

(For further discussion, see text)

	EXPERIMENTAL			CONTROL			
GAS MIXTURE	DURATION OF EXPOSURE TO GAS, MIN.	CHROMOSOMES COUNTED	PER CENT DICENTRICS AND RINGS	GAS MIXTURE	CHROMOSOMES COUNTED	PER CENT DICENTRICS AND RINGS	PER CENT INCREASE OF DICENTRICS AND RINGS OF EXPERIMENTALS OVER CONTROLS
5 atm. CO + 1 atm. air	60	3000	7.8	5 atm. N_2 + 1 atm. air	3000	8.9	Negligible

cytological level we may inquire whether CO causes an increase in the initial frequency of x-ray induced breaks, or affects the reunion of broken ends such that recombination is favored over restitution or both. On the molecular level we may inquire as to the biochemical action of CO on the microspores. Let us first consider the cytological question.

The experimental results thus far presented provide no basis for deciding whether CO acts on the initial frequency of chromosome breakage or on the recovery process. To this end use was made of Lea's calculation that the average time elapsing between breakage and restitution in Tradescantia chromosomes was four minutes.[11] If CO exerted its effect on the recovery process, then it should be possible to affect the yield of chromosome aberrations by exposing inflorescences to CO within this four-minute period. The results of experiments carried out to test this point are presented in table 3. Here the inflorescences were compressed with 5 atmospheres of CO or N_2 within 30 seconds after irradiation. In view of the

absence of effects, it seems evident that CO in some way exerts its action on the breakage process itself. This conclusion finds analogy in the conclusions of Giles and his coworkers on the effects of oxygen during irradiation. They suggest that "the average restitution time for broken ends is the same in the presence and in the absence of oxygen" and that "oxygen exerts its effect by increasing the initial frequency of breaks rather than by modifying the behavior of broken ends during the recovery process."[8]

We may now inquire as to the mode of action of CO within the microspores. Analogous effects have been extensively studied in yeast, in animal tissues and, to a certain extent, in the tissues of plants (Lemberg and Legge,[12] Warburg,[13] and Bhagvat and Hill[14]). There is a substantial evidence that only one group of substances is affected by CO in living systems (exclusive of certain bacteria). These are the heavy-metal-containing enzymes, such as cytochrome oxidase, certain polyphenol oxidases and certain peroxidases. The role of these enzymes in plants and their sensitivity to CO is described in the three references listed above and in papers by Okunuki[15] and Minima and Tylkina.[16]

Okunuki[15] has shown that CO and KCN inhibited the respiration of the pollen of *Lilium* and that the CO inhibition is reversed by light. This last fact gives assurance that cytochrome oxidase is the enzyme affected by CO, since part of the definition of cytochrome oxidase includes light reversibility of CO inhibition. Moreover, this same investigator demonstrated spectroscopically the presence of absorption bands of cytochromes *a*, *b* and *c* in whole pollen and suggested that the cytochrome system played a fundamental role in the normal metabolism of pollen. Since Tradescantia is also a member of the Order Liliales, it seems likely that in Tradescantia microspores the CO acts by inhibiting cytochrome oxidase and that the increased chromosome aberration is a consequence of this inhibition. This suggestion is supported by the observation that oxygen can reverse the CO inhibition (see table 1)—a further distinguishing property of cytochrome oxidase.

The results of the present study may be compared to advantage with other experiments in the literature. Our experiments have shown that CO alone does not cause chromosome breaks. However, other cytochrome poisons, *per se*, such as sodium azide (Wyss, *et al.*[17] on *Staphylococcus*) and KCN (Wagner, *et al.*,[18] on Neurospora) have been shown to cause mutations. It is probable that these agents are exerting their mutagenic effect, not by their action on the cytochrome, but by inhibiting catalase (Sparrow[4]). This is presumed to cause an accumulation of organic peroxides which in some way are thought to be responsible for chromosome breaks and mutations. It is, therefore, noteworthy that CO does not inhibit catalase and consequently does not cause chromosome aberrations in so far as we have been able to determine.

The action of KCN on irradiation has been studied by D'Amato and Gustafsson[19] on barley seeds pretreated with KCN for 24 hours. They found an *increase* in visible mutation rate with low concentrations of KCN (10^{-3} *M*, 10^{-4} *M*), but a *decrease* in visible mutations with 10^{-2} *M* KCN. This apparent inconstancy can be removed if one notes that in the same paper they reported a decrease in mutation rate at high pH (pH 10). Since 10^{-2} KCN has a pH of about 10.5, one may suggest that this high concentration of KCN was exerting its effect in its capacity as a base, rather than as a specific metabolic inhibitor. With this consideration the results of D'Amato and Gustafsson do not conflict with our findings. Moreover, while it is true that KCN is a cytochrome poison by virtue of its heavy-metal-complexing capacity, it is also an effective carbonyl binding agent reacting with many vital substrates (e.g., oxalacetate, pyruvate, etc.). It is thus somewhat difficult to compare results obtained with this relatively non-specific agent (KCN) with results obtained with an agent whose biochemical target is well defined (CO). Therefore, only with reservations do we suggest that KCN acts on x-ray induced mutations in the same way that CO acts on x-ray induced chromosome aberrations—namely, by inhibiting the cytochromes. We are at present studying the effects of HCN gas on x-ray induced chromosome aberrations in Tradescantia.

Since CO acts on the oxygen utilizing part of the metabolic machine, the influence of oxygen *per se* on radiation effects is worth examining. As was noted in the introduction, oxygen has been shown to cause an increase in chromosomal aberrations. We have confirmed this. However, in our experiments the effects of even four atmospheres of oxygen are small compared to effects of CO (see table 1). Notwithstanding, we are confronted with the striking fact that while oxygen alone and CO alone increased aberration frequency, when the two gases were applied simultaneously the CO effect seemed to disappear. The aberration frequency under such circumstances was only slightly increased and was the same as if oxygen alone had been applied (table 1).

To explain this phenomenon we considered the possibility that inhibition of cell respiration by CO caused an *increase* in intra-cellular oxygen tension, and that this in turn caused an increase in chromosome aberrations. However, since even extremely high pressures of oxygen did not exert an appreciable effect in our experiments we have discounted this possibility. Another explanation was suggested by the detection of Okunuki[15] of flavoproteins in *Lilium* pollen. These non-metallo-enzymes can transfer electrons to the cytochromes which then react with molecular oxygen yielding H_2O. However, some flavoproteins can also react directly with molecular oxygen and in so doing yield H_2O_2 as their normal end product (Lardy[20]). It is probable that when the cytochromes are inhibited the

flavoproteins act as terminal oxidases. This could bring about an accumulation of H_2O_2 which would sensitize the chromosomes to irradiation.

It is also worth noting that while cytochromes work at full capacity at low oxygen tensions (*ca.* 4 mm. Hg), flavoprotein activity increases with oxygen tensions at least as high as 760 mm. Hg (one atmosphere) (James and Beevers[21]). With high oxygen tensions we may therefore expect greater flavoprotein activity and consequently more H_2O_2. This would explain the observation of Conger and Fairchild[10] that pure oxygen sensitizes Tradescantia microspores to irradiation and can in fact *per se* produce chromosomal aberrations.

The final consideration is whether the CO is acting directly on the nucleus or indirectly via the cytoplasm. The status of enzymes in the nuclei of cells is not as well defined as that of the cytoplasm and the cytoplasmic granules and this question must be left unanswered for the moment. However, there is one piece of data presented in this paper which suggests that the CO is acting on the cytoplasm. This is the fact that it requires one hour for the CO to exert its full effect in increasing chromosomal aberrations (Fig. 1). It is possible that this delay is due to the time it takes the changes brought about in the cytoplasm by CO to affect the nucleus. Differential centrifugation and direct spectroscopic examination of separated parts of the microspores might yield some direct evidence on the presence of a CO-heavy-metal complex in the nucleus or cytoplasm.

In conclusion we tentatively suggest the following explanation for some of the known effects of oxygen and CO on chromosome aberrations. Our basic premise (admittedly one that is not universally accepted) is that chromosome aberrations closely follow H_2O_2 production.

We believe that CO affects a heavy-metal-containing enzyme in the microspores, probably cytochrome oxidase, in the cytoplasm, nucleus, or both. Furthermore, we suggest that when cytochrome oxidase is inhibited by CO, the flavoproteins act in a greater measure than usual as terminal oxidases, producing large amounts of H_2O_2. This H_2O_2 sensitizes the chromosomes to irradiation. We believe that oxygen, on the other hand, affects the flavoproteins in the microspores: low oxygen tensions decrease their activity as terminal oxidases and this reduces the production of H_2O_2; high oxygen tensions increase their activity, and this increases the production of H_2O_2. Oxygen when applied simultaneously with CO almost completely reverses the effect of CO on chromosome aberrations presumably by reversing the CO-inhibition of cytochrome oxidase.

We would like to emphasize that these suggestions do not preclude the possibility of alternative actions of O_2 during irradiation such as aiding in the production of activated water, etc. However, it seems that in a large measure oxygen exerts its action by altering the biochemical processes of the cell. At present experiments are being carried out to determine the

effects of CO and oxygen on x-ray induced mutations in Drosophila and on x-ray induced injury to cells in tissue culture. It is hoped that these experiments will give some additional clues as to the relation between the metabolic activity of cells and their sensitivity to irradiation.

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† We wish to thank Professor Carroll M. Williams for helpful suggestions and for reading the manuscript.

‡ The slightly elevated aberration frequency that remains when CO and O_2 are simultaneously present at equal positive pressures has been shown to be due to O_2 alone, and not to incomplete reversal of the CO-cytochrome oxidase complex.

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STUDIES ON A SUPPRESSOR OF NON-ALLELIC ACETATE-REQUIRING MUTANTS OF NEUROSPORA*

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In 1947, Houlahan and Mitchell[1] reported the first instance of a suppressor mutation in *Neurospora* although such a phenomenon had previously been observed in *Drosophila*. In the case which they reported, a mutation arose which resulted in suppression of a pyrimidine requirement in three mutants presumed to be allelic but not in two demonstrated as non-allelic to these.

The present study deals with a spontaneously occurring suppressor mutation which has as its effect partial removal of the growth requirement for three non-allelic *Neurospora* mutants which will grow when supplied with acetate. In a previous study,[2] it was hypothesized that acetate is synthesized through two pathways, the main one being blocked by gene mutations in the class of mutants studied, and the other pathway blocked by some product of glucose metabolism. The secondary pathway becomes operative when certain carbon sources are used and leads to the synthesis of limited quantities of acetate. In the present report, it is suggested that the suppressor gene is responsible for opening the secondary pathway of acetate formation which ordinarily is inoperative due to an inhibition phenomenon.

Genetic Studies on Mutants.—Three mutants, designated S-34, S-210 and S-48, were isolated in independent mutant hunts by the method of Lein, Mitchell and Houlahan.[3] These mutants behaved similary under a variety of conditions and as yet no distinguishing physiological characteristics have been found Various growth characteristics of these mutants have been reported previously.[2, 4] In general, the manipulative techniques used in the studies to be reported were those described by Beadle and Tatum.[5] When crossed to wild type, the mutants yielded segregations indicating that the acetate requirement was associated with a single gene mutation. Good heterocaryons resulted when each mutant was grown in combination with the other on minimal medium. In addition to this indication of non-allelism, genetic studies were also undertaken. Preliminary attempts to cross the mutants on acetate-supplemented Westergaard medium[6] proved unsuccessful. After various modifications gave no satisfactory results, it was found that fertile perithecia could be obtained when heterocaryons, which were formed on minimal medium, were inoculated on Westergaard medium and conidia of one of the members of the heterocaryotic pair applied, a procedure suggested by Beadle and Coonradt.[7] The

appearance of wild type in ascus dissections, as shown in Table 1, was considered to be proof of non-allelism of the two members of the heterocaryon. Control inoculations of each member of the heterocaryotic pair into minimal medium were always carried out to check the absence of wild type conidia that may have arisen through back mutation. Brackets in the table indicate members of the heterocaryotic pair. Since all crosses yielded some wild type, it could be concluded that the three mutants were non-allelic.

To check the dissections, a back cross was carried out with one of the mu-

TABLE 1

CROSSES BETWEEN HETEROCARYONS AND MEMBERS OF HETEROCARYOTIC PAIRS

CROSS	ASCI WITH NO WILD TYPE SPORES	ASCI WITH ONE PAIR WILD TYPE SPORES	ASCI WITH TWO PAIRS WILD TYPE SPORES	TOTAL ASCI DISSECTED
(S-34a + S-210a) × S-34A	0	7	0	7
(S-34a + S-210a) × S-210A	2	6	1	9
(S-34a + S-48a) × S-48A	2	8	0	10
(S-34a + S-48a) × S-34A	4	3	2	9
(S-48a + S-210a) × S-48A	5	7	6	18

TABLE 2

CROSSES TO DETERMINE PRESENCE OF SUPPRESSOR

CROSS	ASCI WITH 2PRS. WT SPORES, 2 PRS. SP SPORES	ASCI WITH 2 PRS. M SPORES, 2 PRS. WT SPORES	ASCI WITH 2PRS. WT SPORES, 1 PR. M SPORES, 1 PR. SP SPORES	TOTAL ASCI DISSECTED
I5 × S-34a	4	3	7	14
I3A × S-34a	0	14	0	14

WT = Wild Type
Sp = Suppressed
M = Mutant

tants from the cross (S-48a + S-210a) × S-48A which was presumed to be a double mutant since it came from an ascus containing two pairs of mutant and two pairs of wild type ascospores. It was crossed to wild type and of eleven asci which germinated, six contained six mutant and two wild type spores, four had four mutant and four wild type spores and one had eight mutant spores, demonstrating that the presumed double mutant had indeed two mutant loci.

Genetic Studies on the Suppressor Gene.—It was observed that certain cultures of previously stable mutants grew on minimal medium, although not to the extent ordinarily obtained with wild type. To check for back mutation, one such culture arising from S-34 was crossed to wild type. Of

eleven asci dissected, germination was complete in four. In each case a distribution of two mutant, four wild type and two "leaky" spores was noted. Those designated temporarily as "leaky" gave approximately one-fourth the normal wild type growth. These results suggested the formation of a suppressor gene in the culture of S-34 by a spontaneous mutation at a gene locus other than that responsible for the acetate requirement. To test the hypothesis, a member from each pair of wild type in one ascus, designated I-3 and I-5, was crossed to S-34. Theoretically, one such pair should contain the suppressor gene and the other should not. The result of such a

TABLE 3

CROSSES OF 15A TO MUTANTS NON-ALLELIC TO S-34

CROSS	ASCI WITH 2PRS. WT SPORES, 2 PRS. SP SPORES[a]	ASCI WITH 2 PRS. M SPORES, 2PRS. WT SPORES	ASCI WITH 2PRS. WT SPORES, 1 PR. M SPORES, 1 PR. SP SPORES	TOTAL ASCI DISSECTED
15A × S-210a	2	2	9	13
15A × S-48a	5	6	7	18

[a] WT = Wild Type. Sp = Suppressed. M = Mutant

TABLE 4

GROWTH OF MUTANTS WITH AND WITHOUT THE SUPPRESSOR GENE IN MEDIA CONTAINING GLUCOSE OR GLYCEROL AS THE CARBON SOURCE

MUTANT	4-DAY WEIGHT IN MG. FRIES + GLUCOSE	FRIES + GLUCOSE + NA ACETATE (25 MG.)	FRIES + GLYCEROL	FRIES + GLYCEROL + NA ACETATE (25 MG.)
S-210	0.0	34.8	5.4	16.4
S-210 + Sp	4.6	18.5	4.3	15.5
S-48	0.0	27.3	7.6	11.9
S-48 + Sp	7.1	26.7	7.2	15.1

cross is shown in Table 2 and it confirmed the presence of a suppressor gene. It will be seen from Table 2 that I-5 contained the suppressor gene, since the I-3 cross resulted in typical 4 to 4 segregation of mutant and wild type characters.

The question next arose as to whether the suppression was specific for S-34 or whether it would also act upon the non-allelic mutants S-210 and S-48. The results of crosses made to each of these are given in Table 3. As seen in the table, the suppressor obtained from a culture of S-34 is nonspecific and can be demonstrated to partially relieve the acetate requirement for S-210 and S-48 as well as S-34.

Biochemical Aspects of Suppressor Action.—On the basis of the work previously mentioned, and the observation that the acetate requirement was only partially relieved by the suppressor gene, it was thought that the suppression could perhaps be explained as a new mutation responsible for the opening of the secondary pathway of acetate synthesis that had been previously postulated.[2] The existence of the secondary pathway was

postulated primarily because if a carbon source such as glycerol is used, one obtains a small amount of growth with these mutants without any acetate in the medium, indicating that acetate can be formed in limited quantities from a carbon source such as glycerol. Addition of small quantities of glucose abolishes this growth and it was therefore thought that this secondary pathway is susceptible to inhibition by some product of glucose metabolism.

To test the hypothesis that the suppressor gene permits limited acetate synthesis through opening of the pathway ordinarily blocked by the presence of glucose, an experiment was carried out in which mutants with and without the suppressor gene were grown on media containing glucose or glycerol as carbon sources both with and without a supplement of acetate. The general techniques used in the growth studies are described elsewhere.[5] Results obtained using mutants S-48 and S-210 are presented in Table 4.

It will be seen from the table that while the mutant strains carrying the suppressor gene grow to a certain extent on unsupplemented medium with glucose as a carbon source, when grown with glycerol as a carbon source they do not show any increased weight over that of the mutant without the suppressor gene. This demonstrates that the suppressor effect is not additive to the growth one obtains with glycerol as a carbon source and suggests that the suppressor operates through the same pathway of acetate production which functions when glycerol is the carbon source. Since this pathway produces only limited amounts of acetate, the growth that is obtained on minimal medium in the presence of the suppressor gene is small and markedly increased by supplementing the medium with acetate as shown in the table.

Discussion.—Since growth of the mutants in minimal medium is obtained with the suppressor gene in the presence of glucose, a possible mode of its action would be the relief of the inhibition produced by glucose. A demonstration of this must await the finding of the precise biochemical block produced by the glucose metabolite.

Although the three mutants studied have been shown to be non-allelic, it is not known whether the three mutations have caused a blocking of the same reaction in carbohydrate metabolism or whether separate reactions are blocked. Thus, if the gene mutations are involved in the blocking of the synthesis of a necessary cofactor for an enzymatic reaction involved in acetate formation, the same reaction would be blocked in all three cases. The hypothesis presented would be suitable to explain the results whether the same or different reactions are blocked by the gene mutations since it is postulated that a pathway of acetate production usually inoperative under the conditions of experimentation, becomes operative due to a mutation at another gene locus.

The finding of the suppressor mutation raises the question of the preva-

lence of such phenomena. When one finds that a biochemical mutant has reverted to wild type characteristics, it is generally assumed that a back mutation at the previously mutated locus has occurred. This is especially the case when working with bacteria where genetic techniques are so limited. It may be that suppressor formation is quite prevalent. In our laboratory after finding the reported suppressor, a small-scale experiment was set up and another suppressor for one of the other acetate mutants was demonstrated. If suppression of many different biochemical requirements can be demonstrated to occur with a marked frequency, it might be an indication of still another versatile characteristic of the gene supply. There may possibly be many genes which do not have an essential function but which may serve as a pool of genic material which on mutation make survival more probable under certain conditions of selection.

Summary.—Three mutants of Neurospora requiring acetate for growth were shown to be non-allelic by genetic and heterocaryon tests. A suppressor mutation which partially relieves the acetate requirement arose in one of the strains and it was demonstrated that it would also suppress the acetate requirement for the other non-allelic mutants. Evidence was presented indicating that the suppressor mutation opened up a secondary pathway of acetate production.

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*CAROTENOID DIFFERENCES IN TOMATOES**

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In an earlier paper[1] carotenoid differences in red, tangerine and yellow genotypes (*RRTT*, *RRtt*, *rrTT*) of *Lycopersicon esculentum* were discussed. We have now completed analyses of two independent sets of crosses involving the three possible red-fruited heterozygotes (*RrTt*, *RRTt* and *RrTT*).

The double recessive *rrtt* has also been isolated as a segregant from *RrTt*. In addition, data on the hybrid between the double recessive and yellow (*rrtt* × *rrTT* → *rrTt*) are included.

Experimental Strains.—Two strains of red, numbers 434 and 622, of Mexican origin were selected. In both, the fruit was smaller than in commercial "Bonny Best," and the pigment content somewhat higher, but qualitatively they were indistinguishable. A tangerine, number 165, was of the "Jubilee" strain. The yellow, number 378, contained in addition the following recessive genes as markers: dwarf, oval, peach, compound inflorescence. All except 165 contained the alkali-soluble skin pigment gene.

TABLE I

MICROGRAMS OF CAROTENOID PER GRAM OF FRUIT

	REDS—*RRTT*, *RrTt*, ETC.	TANGERINE—*RRtt*	YELLOWS—*rrTT* AND *rrTt*	DOUBLE RECESSIVE—*rrtt*
Lycopene set	70–130[a]	40–55[b]	0–0.5[c]	10–15[d]
ψ-carotene	...	8–15	...	...
ζ-carotene	0–0.1	20–50	...	0–0.1
β-carotene	5–10	3–12	1–3	0.5–1.0
Phytofluene	3–5	4–7	*Ca.* 0.1	0.7–1.0
Total	80–150	75–150	3–7	15–20

[a] 90–100% *all-trans* lycopene.
[b] 50–80% prolycopene, remainder *mono-cis* isomers.
[c] 100% *all-trans* lycopene.
[d] 40–70% prolycopene, remainder *mono-cis* isomers.

The heterozygous reds, *RrTT* and *RRTt*, were obtained in the F_1 hybrids of red × yellow and red × tangerine. The F_1 from 378 × 165 gave the remaining red *RrTt*. From plantings of F_1 and F_2 generations of the last mentioned, the double recessive isolates, were obtained. Two of these were crossed to 378. Genetic analysis of the F_2 and F_3 populations are discussed elsewhere.

Carotenoid Analyses.—Extraction and analytical procedures have already been described.[1] To facilitate comparisons, we have used the absorption coefficients listed by Porter and Lincoln.[2] Additional computations were made from spectroscopic measurements of crude extracts as follows:

1. Total lycopene (i.e., all isomeric forms, including prolycopene) in reds, tangerines and double recessives: an absorption coefficient of 224 at 495 mμ in petroleum ether was used for the iodine-catalyzed mixtures. Curves for the mixtures obtainable from all-*trans* lycopene, crude extracts of the reds, tangerines and double recessives are superimposable from 510 to 485 mμ. Since the total concentration of lycopene isomers in a given

solution is constant, whether iodine-catalyzed or not, the optical densities (D) and absorption coefficients (k) are related to the concentration, c thus:

$$c = D^b_{502}/k^b_{502} = D^a_{495}/k^a_{495},$$

where the superscripts b and a refer to conditions before and after catalysis, and subscripts 502, 495 to the wave-length. Whence

$$k^a_{495} = k^b_{502} \times D^a_{495}/D^b_{502} = 320 \times 0.7 = 224.$$

2. Total carotenoid in yellow genotypes: This is calculated on the more arbitrary assumption of a coefficient of 230 at 465 mμ, the position of the first absorption band. Although β-carotene is usually the most abundant single component, appreciable quantities of strongly adsorbed xanthophylls cause the maxima for the extract to be shifted to 465 and 440 mμ. Some yellow strains are very weakly pigmented, and large quantities of fruit would be necessary for adequate identification of the numerous minor components. Lycopene is at most a minor contributor, so that the arbitrary assumption of an average coefficient is not unreasonable.

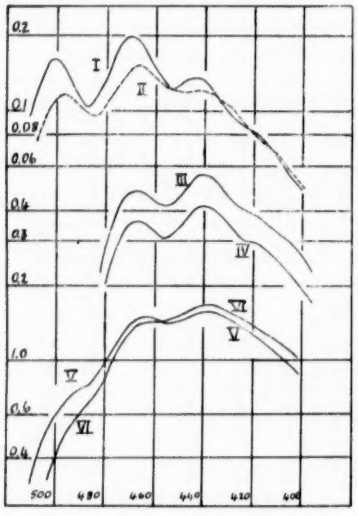

FIGURE 1

Abscissa, wave-length, ordinate, optical density (in arbitrary units). Curve *I*, extract of *RRTT*, in petroleum ether; *II*, iodine-catalyzed aliquot of *I*; *III*, *rrTT*; *IV*, *rrTt*; *V* and *VI*, *rrtt*. *VI* has a higher proportion of prolycopene in relation to other lycopene isomers than is the case with *V*.

Results.—Results are summarized in table 1 for the different genotypes. The heterozygous reds were qualitatively indistinguishable from the homozygous red, both spectroscopically (Fig. 1, curves *I* and *II*) and chromatographically. β-carotene fractions separated on MgO-SiO_2 columns were also spectroscopically identical. The lycopene content for *RRTT* samples averaged 80 μg in 1950 and 130 μg per gram in 1951. The heterozygous reds varied irregularly from 69 to 100 μg. Fruit size probably contributed to this variability. The *R* and *T* genes are therefore dominant, i.e., no additive effect of *RR* over *Rr* in production of a given pigment was observed.

The yellow *rrTt* is indistinguishable from *rrTT*, (Fig. 1, curves *III* and *IV*).

The double recessive (*rrtt*) has more total pigment than the yellow (*rrTT*), and prolycopene is normally the main pigment (Fig. 1, curves *V* and *VI*). A specimen left in the laboratory for 12 days had less total lycopene than samples determined on harvesting, 11 vs. 13 μg pigment per gram, but whereas the prolycopene content fell from 8 to 5 μg, the content of mono-*cis* isomers rose from 5 to 6 μg. This was reflected spectroscopically in an increase in the absorption at 495 mμ relative to that at 435 mμ, cf. curves *V* and *VI*.

The double recessive also synthesizes more lycopene isomers and more phytofluence than the yellows, while the β-carotene content is definitely less.

One specimen of the remaining genotype, a tangerine heterozygous at the *R* locus, *Rrtt*, has been examined, but not critically analyzed. It was assumed to be a typical tangerine, containing inter alia, *poly-cis* ψ- and ζ-carotenes. Since F_3 segregants included a double recessive, the genotype was clearly *Rrtt*. The pigment content is almost certainly comparable with *RRtt*.

Discussion.—We have not detected *all-trans* lycopene in tangerine stock, or ψ-carotene in red, yellow or double recessives. ζ-carotene may be present in trace amounts in our red specimens. Zscheile and Porter[3] have on occasion found ζ-carotene in considerable quantity in red fruit, but it does not have to be present in measurable amount for normal red pigmentation. Attempts to determine whether a particular component is completely absent or present in trace amounts are not particularly useful here. The same argument applies with respect to *all-trans* lycopene in tangerine (*RRtt*). We separated four minor bands adsorbed above prolycopene, none of which had an absorption maximum beyond 498 mμ in petroleum ether, so that we have no direct evidence for the *all-trans* form. These points do not affect the validity of the data of table 1. The question now arises as to the roles of *R* and *T* genes.

Syntheses of components is given by the following sequences, in increasing order:

Lycopene set	(*rrTT* *rrTt*)	<	*rrtt*	<	(*RRtt* *Rrtt*)	<	(*RRTT, RRTt* *RrTt, RrTT*)	
β-carotene	*rrtt*	<	(*rrTT* *rrTt*)	<	(*RRtt, RRTT, RRTt* *Rrtt, RrTt, RrTT*)			
Phytofluene	(*rrTT* *rrTt*)	<	*rrtt*	<	(*RRTT, RRTt* *RrTt, RrTT*)	<	(*RRtt* *Rrtt*)	

The failure of the tangerine to synthesize as much total lycopene as the red is offset by accumulation of ζ- and *poly-cis* ψ-carotenes, and slightly more phytofluene.

R clearly favors carotenoid synthesis, a view expressed by Went, Le-

Rosen and Zechmeister.[4] It is much more difficult to assign a role to *T*. When this gene is recessive, prolycopene is synthesized regardless of *R*. If we concur with Porter and Lincoln's view[2] that *poly-cis* ψ- and ζ-carotenes are tetra- and octahydrolycopenes, a possible role is seen in the dehydrogenase system. When carotenoid synthesis is great, as in *RRtt*, there is a partial failure of the dehydrogenase, and *poly-cis* ψ- and ζ-carotenes accumulate.

Porter and Lincoln[5] have introduced a gene, *B*, from *L. hirsutum* into *L. esculentum* which markedly affects production of β-carotene, apparently at the expense of lycopene. Before assigning roles to *R* and *T* genes (which conceivably may be quite indirect in their effect on carotenoid synthesis,) it seems to us of critical importance to know what happens at the *R* and *T* loci in the presence of *B*. We have therefore crossed *L. hirsutum* with yellow and tangerine strains, and we hope in due course to report on these hybrids.

Summary.—The carotenoid contents of heterozygous red-fruited tomatoes, of heterozygous yellows and of the double recessive have been compared with the original strains. The double recessive synthesizes prolycopene and also more total carotenoid than the yellow, but far less than the red or tangerine which are approximately equivalent in this respect.

* Presented at the XII International Chemical Congress, New York, N. Y., September, 1951.

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ON CERTAIN DIOPHANTINE EQUATIONS IN RINGS AND FIELDS

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Consider the conditional equation

$$f(x_1, \ldots, x_s) = 0, \qquad \text{(A)}$$

where f is a polynomial in the x's with coefficients in a ring R containing a unity element and the problem of determining the x's as quantities in R. If R is the ring of rational integers or the field of rational numbers, this is a problem in ordinary Diophantine analysis. We first note some

results which are obtained by the use of certain identities in rings. Bouniakowsky[1] noted that (using his notation)

$$x^m X^n + y^m Y^n = z^m Z^n \tag{1}$$

is satisfied identically by, where now R is a commutative ring with a unity element,

$$x = a^\alpha,\ y = b^\alpha,\ z = c^\alpha, \qquad X = b^\beta c^\beta,\ Y = a^\beta c^\beta,\ Z = a^\beta b^\beta,$$

where α, β are rational integers such that $m\alpha - n\beta = 1$, m and n are relatively prime, and $c = a + b$ with a and b arbitrary elements of R. Also a similar relation may be satisfied involving any number of unknowns. Ward[2] showed that the equation, F homogeneous of degree n,

$$F(x_1, x_2, \ldots, x_s) = y^k;\ (n, k) = 1, \tag{2}$$

F having coefficients ϵR, is solvable identically, where the solutions $x_1, \ldots, x_s, y$ are certain polynomials with coefficients ϵR. If R is a field, he showed that all solutions of (2) are given by this method; but if, for example, R is the ring of rational integers, then this is not the case. For example, the solutions or the quantities $x_1, x_2, \ldots, x_s, y$ will in general have a common divisor.

We now consider the equation

$$x_1^{m_1} + c_2 x_2^{m_2} + \ldots + c_s x_s^{m_s} = 0, \tag{3}$$

where the c's are in R and $m_i > 0$; $i = 1, 2, \ldots, s$; $c_1 \ldots c_s \neq 0$. We suppose further that $(m_1, m_2 m_3 \ldots m_s) = 1$. There exist positive integers λ and μ such that

$$\lambda m_1 - \mu m_2 \ldots m_s = 1. \tag{4}$$

Let

$$y_1 = -c_2 y_2^{m_2} - \ldots - c_s y_s^{m_s},$$

then (3) is satisfied identically by

$$x_1 = y_1^{\lambda},\ x_2 = y_1^{\mu A/m_1 m_2} y_2,\ x_3 = y_1^{\mu A/m_1 m_3} y_3,\ \ldots,\ x_s = y_1^{\mu A/m_1 m_s} y_s$$

with $A = m_1 m_2 \ldots m_s$, and the y's in R. This method may be shown to give all the solutions of (3) in the case where R is a finite field, by proceeding as in another paper[3] and selecting λ and μ to be prime to $(p^n - 1)$, where p^n is the order of the field. In the case of a general ring we may have common divisors as we mentioned in connection with (2).

We know many results concerning the number of solutions of conditional equations in finite fields. Lacking formulas for the roots, however, we may take up methods for determining functions of the roots.

Consider the equation (A) and suppose that the coefficients and the x's are quantities in a finite field $F(p^n)$; write $f = f(x_1, x_2, \ldots, x_s)$. Then the right hand member of

$$N(m_1, \ldots, m_s) = \sum (x_1^{m_1} \ldots x_s^{m_s} - x_1^{m_1} \ldots x_s^{m_s} f^{k(p^n-1)}), \quad (5)$$

where each x ranges independently over the non-zero elements of $F(p^n)$, reduces, for $m_i \geqq 0$, $i = 1, 2, \ldots, s$, to

$$\sum r_1^{m_1} \ldots r_s^{m_s}$$

for every set of values $(r_1, r_2, \ldots, r_s)$ such that $f(r_1, \ldots, r_s) = 0$ and the whole sum becomes zero in the field otherwise,[4] since $f^{p^n-1} = 1$ in the latter case. Hence

$$N(m_1, \ldots, m_s) = \sum r_1^{m_1} \ldots r_s^{m_s}. \quad (5a)$$

If we confine ourselves to equations of the type

$$c_1 x_1^{m_1} + \ldots + c_s x_s^{m_s} = c, \quad (6)$$

where the coefficients and the x's belong to a finite field, we can also employ the methods of cyclotomy to such problems. In what follows we shall give a number of results concerning not only the number of solutions of (6), but also theorems concerning functions of the roots. We hope to be able to publish full proofs elsewhere.

In (6) let the c's be elements of a finite field $F(p^n)$ of order p^n where p is prime, $c_1 \ldots c_s \neq 0$ in $F(p^n)$; and $p^n - 1 \equiv 0 \pmod{m_i}$, $i = 1, \ldots, s$. Denote by $[c_1, \ldots, c_s; c]$ the number of distinct sets of solutions of (6) in $F(p^n)$; also denote by $(c_1, \ldots, c_s; c)$ the number of distinct sets of non-zero solutions of (6) in $F(p^n)$.

Let g be a multiplicative generator of the non-zero elements of $F(p^n)$ and $\alpha_i = e^{2\pi i/m_i}$. If ind a_i is defined such that $g^{\text{ind } a_i} = a_i$, then

$$(c_1, \ldots, c_s; c) = \sum_{u_1, \ldots, u_s} \psi_c(u_1, \ldots, u_s) \prod_{i=1}^{s} \alpha_i^{-u_i \text{ ind } c_i}, \quad (7)$$

where the u's range independently over $0, 1, \ldots, m_i - 1$; $i = 1, \ldots, s$; and

$$\psi_c(u_1, \ldots, u_s) = \sum_{a_1 + \ldots + a_s = c} \prod_{i=1}^{s} \alpha_i^{u_i \text{ ind } a_i}, \quad (8)$$

$s > 1$ when $c = 0$;

$$\psi_0(u_i) = 0 \quad (8a)$$

and each a ranges over the non-zero elements of $F(p^n)$.

Denote by $\Phi_c(c_1, \ldots, c_t)$ the number

$$\sum_{u_1, \ldots, u_t} \psi_c(u_1, \ldots, u_t) \prod_{i=1}^{t} \alpha_i^{-u_i \text{ ind } c_i}, \quad (9)$$

where the u's range independently over the sets $1, \ldots, m_i - 1$; $i = 1, \ldots, t$. Also denote by

$$S_t^s F(b_1, \ldots, b_t) \tag{10}$$

the sum of the F's of the $\binom{s}{t}$ distinct sets of b's taken t at a time, where F is an arbitrary function of $(b_1, \ldots, b_t)$. We find the

THEOREM I. *If $s > 1$,*

$$(c_1, \ldots, c_s; c) = p^{(s-1)n} + \gamma - \sum_{t=1}^{s-1} S_t^s (c_1, \ldots, c_t; c) + \Phi_c(c_1, \ldots, c_s), \tag{11}$$

where $\gamma = -1$ if $c = 0$, but $\gamma = 0$ for $c \neq 0$, $(c_1, \ldots, c_s; c)$ denotes the number of sets of non-zero solutions of (6), and where the other symbols are defined in (9) and (10).

This result gives:

COROLLARY.[5] *If $s > 1$, then*

$$[c_1, \ldots, c_s; c] = p^{(s-1)n} + \Phi_c(c_1, \ldots, c_s), \tag{12}$$

where the left-hand member is the number of sets of solutions of (6).

When $c = 0$, (9) can be put in another form by the use of

$$\psi_0(u_1, \ldots, u_s) = \begin{cases} (p^n - 1)\psi_1(u_2, \ldots, u_s)\alpha_1^{u_1 \operatorname{ind}(-1)}, & \prod_{i=1}^{s} \alpha_i^{u_i} = 1. \\ 0, & \prod_{i=1}^{s} \alpha_i^{u_i} \neq 1. \end{cases} \tag{13}$$

By the use of (7) many relations involving the number of non-zero solutions of (6) when $c \neq 0$ have been obtained in various published papers. If we employ (7) for $c = 0$ or (12) and (13), we will usually find similar relations involving $(c_1, \ldots, c_s; 0)$, and $[c_1, \ldots, c_s; c]$. Also using (7) it is possible to obtain in general better limits than given by Hua and Vandiver[5] or by Faircloth[5] for $(c_1, \ldots, c_s; c)$. These formulas are complicated, however.

We now consider the type of functions of the roots of (6) which we considered in (5a). Let

$$\psi' = \psi'(\beta^{t_1}, \ldots, \beta^{t_s}; \alpha_1^{u_1}, \ldots, \alpha_s^{u_s}), s > 1,$$

and

$$\psi' = \sum_{h_1, h_2 \ldots h_s} \prod_{i=1}^{s} \beta^{t_i h_i} \alpha_i^{h_i u_i}; \; t_i \geqq 0, \tag{14}$$

where each h ranges independently over $0, 1, \ldots, p^n - 2$ such that

$$g^{h_1} + \ldots + g^{h_s} = 1.$$

This is a number of the same general type as (8) since we can express each α_i as a power of β. Also (6) can be put in the form, since we are excluding zero solutions for the x's,

$$g^{d_1 + m_1\delta_1} + \ldots + g^{d_s + m_s\delta_s} = 1, \tag{15}$$

where the d's are given and our problem reduces to finding all possible δ_i's in the set $0, 1, \ldots, (m'_i - 1)$ with $m'_i m_i = p^n - 1$, $i = 1, 2, \ldots, s$. Let there be e sets of δ's which satisfy (15); they may be represented by

$$\delta_{1j}, \delta_{2j} \ldots \delta_{sj}$$

with $j = 1, 2, \ldots, e$. Then we find

$$\sum_{u_i} \psi' \alpha_i^{-d_i(t_i + u_i)} = \sum_{j=1}^{e} \prod_{i=1}^{s} \beta^{\delta_{ij} m_i t_i} m_i, \tag{16}$$

where the t's are arbitrary integers. Each u_i ranges independently over $0, 1, \ldots, m_i - 1$; $i = 1, 2, \ldots, s$. This gives the following relation in $F(p^n)$,

$$\sum_{\substack{h_1, \ldots, h_s \\ u_i}} \prod_{i=1}^{s} g^{t_i r_i + m'_i u_i r_i} = \sum_{j=1}^{e} \prod_{i=1}^{s} g^{\delta_{ij} m_i t_i} m_i, \tag{17}$$

where $r_i = h_i - d_i$. The right-hand member of (17) is a function of the solution, $g^{\delta_1}, \ldots, g^{\delta_s}$ in the δ's of (15).

We now consider varying d_1 and m_1 in (16) keeping the other d's and m's fixed. Set $\{d_1, m_1\}$ for the right-hand member of (16). Then we may derive

THEOREM II. *Let $t_1 = 0$, k_1 any divisor of m_1 with $m_1 = k_1 k'_1$, then*

$$\sum_{v=0}^{k'_1 - 1} \{d_1 + vk_1, m_1\} = \{d_1, k_1\}. \tag{18}$$

Since ψ' is symmetric in the m's, we may replace d_1 in the above statement by d_i; $i = 1, 2, \ldots, s$, provided $t_i = 0$.

ADDED IN PROOF: Since writing the above we have found that Aucoin (cf. *Proc. Amer. Math. Soc.*, **2**, 760 (1951) and references there given) found explicit solutions in the ring of rational integers I of many different types of Diophantine equations with coefficients in I. Included in these forms are our (1), (2), and (3), if we restrict our R to I. Also, it appears that some, at least, of his results concerning other types of equations still hold if we replace I by R, a commutative ring with a unity element.

[1] *Bull. Acad. Sci. St. Petersburg*, **6**, 200–202 (1848). He gave this result only for the ring of rational integers, but it obviously holds for R as we define it.

[2] These PROCEEDINGS, **37**, 113–114 (1951).

[3] *Ibid.*, **35**, 486 (1949). However, it is to be noted that in the paper here referred to our problem is of a little bit different type than that which we are considering in the present paper. Although we have formulas for the roots in the latter case, we have no method in general for counting the number of different sets of solutions. The latter problem concerning certain types of equations was also considered by Faircloth, these PROCEEDINGS, **37**, 621–622 (1951). When equation (A) has no constant term, the existence of solutions in a finite field has been considered, using methods quite different from any discussed here, by Chevalley, *Abhandl. Math. Seminar Hamburg Univ.*, **11**, 73–75 (1936); and by Warning in an article which follows it in the periodical mentioned,

pp. 76–83. Cf. also R. Brauer, *Bull. Am. Math. Soc.*, **51**, 749–755 (1945). For calling our attention to the first reference, we are indebted to Dr. Ernst Snapper; for the third, to Dr. H. N. Shapiro.

[4] Cipolla, *Periodico. di Mat.*, **22**, 36–41 (1907). For every m in (5a) equal to zero then N reduces to the number of sets of roots of (A), a formula known to V. A. Lebesgue, for $n = 1$, and this was employed to advantage by S. Schwarz, *Quart. J. Math.*, **19**, 160–163 (1948), in order to obtain a theorem concerning the existence of solutions of (6). Cipolla gave (5a) only for $n = 1, k = 1, s = 1$, but the extension is immediate.

[5] This Corollary together with the formula (7) give a number of solutions of (6) in a finite field $F(p^n)$ with the coefficients in $F(p^n)$ and $c_1, c_2, \ldots, c_s \neq 0$ both for the case where we include zero values for the x's and when we do not. These formulas are all in terms of the ψ numbers defined in (8). For previous references concerning the application of the number (for general n, cf. Stickelberger; Davenport-Hasse, *loc. cit.*)

$$\tau(a^\mu) = \sum_{a \neq k} \alpha^{\mu \operatorname{ind} a} \zeta^{\operatorname{tr}(a)}; \tag{19}$$

where tr stands for trace; k denotes $F(p^n)$; and the closely related ψ number of (8) to the problem of finding the number of solutions of (6) in $F(p^n)$ when n is general, cf. Davenport and Hasse, *J. f. Math.*, **172**, 173–174 (1935), who found (12) for $s = 2$, and its equivalent in terms of (19). Hua and Vandiver, these PROCEEDINGS, **35**, 94–99 (1949) (cf. relations (10) and (11) on p. 97 and the statements above the relations (19) on p. 99), obtained the equivalent of (7) and (12) in terms of (19). Cf. also these PROCEEDINGS, **36**, 146 (relation (8)) (1950). Weil, *Bull. Am. Math. Soc.*, **55**, 497–508 (1949), obtained (12) for $c = 0$ and derived (12) for $c \neq 0$ in a more complicated form. He gave the formulas on pp. 500–502 which show how to express our (8) in terms of (19). Vandiver, these PROCEEDINGS, **36**, 148 (relation (17)) (1950), gave (7) for $c \neq 0$; and Faircloth, these PROCEEDINGS, **37**, 620 (relation (8)) (1951), obtained (12) for $c \neq 0$.

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*A REMARK ON CAVITATIONAL FLOW**

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Recently two independent proofs for the existence of plane cavitational flow have been given by Garabedian and Spencer[1] and by Schiffer which are based on an extremal property of the free streamlines developed formally by Schiffer several years ago. The object of this note is to present an alternate proof, based on the same extremal property, which seems to be quite elementary and to be better suited for generalization to the discussion of axially symmetric cavitational flow. The method used here depends on approximation of extremal curves by extremal polygons with a bounded number of vertices, and it avoids the variations given in terms of conformal mapping which have been used previously to deduce the analyticity of the extremal curves. The method of polygonal approximation

can be applied quite generally, and, in particular, it yields analyticity of the boundary for extremal problems in the theory of mean-univalent functions where the method of interior variations has not met with success.

Let C be a simple closed curve in the z-plane bounding an object B which is symmetric in the x-axis and y-axis and which intersects each vertical or horizontal line in at most one segment. We denote by L a set of curves lying in the exterior of B and in the strip $-k \leq x \leq k$ which bound with C a certain cavity W. We assume that the curves L are symmetric in the x-axis and in the y-axis. The flow past the object $B + W$ bounded by C and L has a velocity potential φ with an expansion

$$\varphi = x + \frac{\alpha x}{r^2} + \ldots, \quad r^2 = x^2 + y^2,$$

near infinity and a normal derivative which vanishes on C and L. We shall term the coefficient α the virtual mass of the flow, and we shall denote by A the area of $B + W$.

For a given positive constant λ, we select the free curves L in such a way that

$$2\pi\alpha - \lambda A = \text{minimum}. \tag{1}$$

Also we denote by L_n the set of polygonal curves with at most $4n$ vertices which have the extremal property (1). It has been shown[1] that the virtual mass α decreases when the object $B + W$ is symmetrized in the x-axis or in the y-axis, while the area A remains unchanged and the object B is invariant (cf. reference 2). Thus for both polygonal curves L_n and general curves L, the extremal object $B + W$ characterized by (1) will be symmetrized.

As the free curves L are stretched to infinity within the strip $-k \leq x \leq k$, the virtual mass α grows at a higher rate than the area A. Since the complex potentials $\zeta = \varphi + i\psi$ of the flows past symmetric objects $B + W$ form in the upper half of the flow regions D a normal family of analytic functions with positive imaginary part, we are able to deduce the existence of bounded extremal curves L or extremal polygons L_n for (1). These curves are monotonic in each quadrant, and hence rectifiable.

In order to analyze the extremal curves for (1), we note that displacement of L a distance δn units along the inner normal with respect to the flow region D yields for A and α the first order shifts

$$\delta A = \int_L \delta n \, ds, \tag{2}$$

$$2\pi\delta\alpha = \int_L \left(\frac{\partial\varphi}{\partial s}\right)^2 \delta n \, ds, \tag{3}$$

according to Hadamard's variational theory. We shall use these formulas only when the shift δn is made on a line segment which is part of L and when δn is either a constant, corresponding to translation of the segment, or a sufficiently smooth function in the interior of this segment. In this case, (2) and (3) are easily derived with complete rigor.

As a first application of (2) and (3), we point out that if the general extremal curves L for (1) contain a segment, then

$$2\pi\delta\alpha = \lambda\delta A \tag{4}$$

for all sufficiently smooth functions δn on the segment, and therefore

$$\left(\frac{\partial\varphi}{\partial s}\right)^2 = \lambda. \tag{5}$$

Hence

$$\frac{\partial\varphi}{\partial x} - i\frac{\partial\varphi}{\partial y} = \text{const.}$$

there, and by analytic continuation we see that the flow is uniform throughout the entire plane. This is absurd, and we conclude that no segments occur on L.

Since the extremal curves L and the extremal polygons L_n are monotonic in each quadrant, we see that every subsequence of L_n has a convergent subsequence which approaches a general set of extremal curves L. In view of the absence of segments on L, it is therefore clear that the maximum length $\epsilon(n)$ of an edge of the polygons L_n must tend to zero as $n \to \infty$.

We now apply (2), (3), and (4) to four symmetrically situated edges of L_n with δn constant. This yields a translation of the edges, and does not alter the number of vertices of L_n. Over any edge l we obtain

$$\int_l \left(\frac{\partial\varphi_n}{\partial s}\right)^2 ds = \lambda \int_l ds, \tag{6}$$

where φ_n is the velocity potential of the flow past L_n and C. If we introduce the analytic function

$$F_n(z) = \frac{1}{\lambda}\int \left(\frac{\partial\varphi_n}{\partial x} - i\frac{\partial\varphi_n}{\partial y}\right)^2 dz,$$

we see by (6) that $F_n(z)$ maps each edge l of L_n on a segment l' of equal length. But since φ_n has a normal derivative which vanishes on $C + L_n$, l' makes with the real axis an angle which is the negative of the angle made by l and the real axis. Therefore, except for a translation, $F_n(z)$ maps L_n segment by segment on its reflection in the real axis.

From the geometry of the mapping, it is now evident that

$$|F_n(z) - \bar{z} + \text{const.}| \leq \epsilon\,(n) \tag{7}$$

on each arc of L_n. If we consider

$$u_n = Re\,\{F_n - z + \text{const.}\},$$
$$v_n = Im\,\{F_n + z + \text{const.}\}$$

as harmonic functions of the complex potential $\zeta = \varphi + i\psi$, we see from (7) that u_n and v_n tend uniformly to zero on each segment of the φ-axis corresponding to L_n. By the theory of normal families, we can select a subsequence $F_k(z)$ of the sequence $F_n(z)$ so that

$$\lim_{k \to \infty} F_k(z) = F(z) = \frac{1}{\lambda} \int \left(\frac{\partial \varphi}{\partial x} - i \frac{\partial \varphi}{\partial y} \right)^2 dz$$

and so that L_k approaches an extremal set of curves L for (1). Since u_n and v_n are bounded uniformly in n outside a neighborhood of infinity, the convergence of u_n and v_n to zero on L_n implies that the limit functions

$$u = Re\,\{F - z + \text{const.}\},$$
$$v = Im\,\{F + z + \text{const.}\}$$

vanish on L. This can best be seen by representation of u_n and v_n in terms of the Poisson integral formula in a suitable plane and passage to the limit. In particular,

$$F(z) = \bar{z} + \text{const., on } L. \tag{8}$$

By Schwarz's principle of reflection, u and v can be continued across the portion of the φ-axis corresponding to L. It follows that

$$z = \frac{1}{2} \{(F + z) - (F - z)\}$$

is analytic as a function of φ along L, and hence L is composed of analytic arcs.

We can now differentiate (8) along L to obtain

$$\left(\frac{\partial \varphi}{\partial s} \right)^2 = \lambda \left| \frac{dF}{dz} \right| = \lambda \left| \frac{d\bar{z}}{dz} \right| = \lambda \tag{9}$$

there. The identity (9) states that L is a constant-pressure free streamline for the flow past $C + L$, and thus our extremal problem (1) leads to the existence of a cavitational flow past B with cavity W.

In conclusion, we point out that the same method of polygonal approximation and geometrical analysis of the mapping $F(z)$ yields an analytic solution of the analogous extremal problem

$$S^3 - \lambda\alpha = \text{minimum}, \qquad (10)$$

where S is the length of the perimeter of the object $B + W$. The free extremal curves L for (10) must be convex, since the convex hull of any arc includes a larger object with greater virtual mass, but with a shorter perimeter. The solution of (10) has by Bernoulli's law a hydrodynamical interpretation in which W represents a pocket of air attached to the body B by a thin free rubber sheet along which pressure forces must be in equilibrium with the forces due to tension. Indeed, Hadamard's variational formulae show that along the free boundary L the square of the velocity is proportiohal to the curvature.

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[1] Garabedian, P. R., and Spencer, D. C., "Extremal Methods in Cavitational Flow," *J. Rational Mechanics Analysis*, to appear shortly.

[2] Pólya, G., and Szegö, G., "Isoperimetric Inequalities in Mathematical Physics," *Ann. Math. Studies*, No. 27, Princeton, 1951.

NUCLEAR STUDIES ON THE MYXOPHYCEAE, A PRELIMINARY REPORT

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Communicated by David R. Goddard, September 28, 1951

Since the observation of Schmitz[1] that the cells of the *Myxophyceae* are differentiated into two regions there have appeared many diverse opinions on the nuclear status of these organisms. In these studies classical cytological methods have been exhaustively utilized by capable investigators. In recent years new methods have evolved from investigations in bacterial cytology. The extreme similarity, in many properties, between the bacteria and the *Myxophyceae* suggested that it might be worth while to apply some of the bacterial cytological methods to the latter. The present paper is a preliminary report on two of the *Myxophyceae*, one filamentous, the other unicellular, which have been studied using bacteriological methods. These two organisms are *Synechococcus cedorum* and *Microcoleus vaginatus*, the former being kindly supplied by Dr. Mary Belle Allen.

Methods.—The organisms were grown in liquid medium, and transferred to agar blocks for fixation. After fixing in osmic acid vapors for 2 minutes, and removing from the agar, the cells were treated with normal hydrochloric acid at 60°C. for 10 minutes. They were stained for 20 minutes with a 1:60 dilution of Giemsa's stain (Harleco) in distilled water. This staining procedure is that recommended by Piekarski[2] for bacteria.

Since the organisms are easily removed from slides by the hydrochloric acid it was found convenient to carry out the acid treatment in 3-ml. centrifuge tubes, and to wash the cells with tap water by centrifugation. The cells can then be smeared on a slide and stained. Greater detail concerning cultivation and processing will be presented in a later communication when a more complete study has been made.

Results.—It appears from this preliminary study that there is a nuclear structure in both organisms studied, and that the organization in both is similar. There appear to be three conditions, an oriented rod-like arrangement, a net-like arrangement and a condensed stage. These stages re-

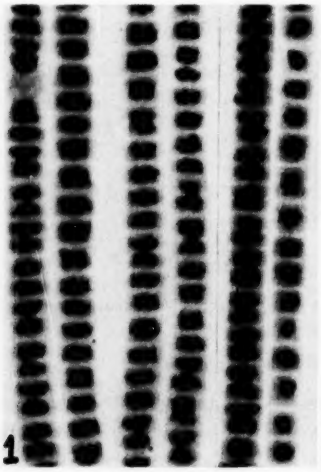

FIGURE 1.

Microcoleus vaginatus sp. Stained as described in the text.

FIGURE 2.

Synechococcus cedorum. Stained as described in the text.

semble to a certain extent the nuclear transformations seen during mitosis in higher plants. In *Microcoleus*, transverse walls can be seen impinging against the fascicles of chromatinic material at the points of constriction.

The heavily stained structures seen in the two organisms gave a positive Feulgen reaction, staining a light to medium violet. These structures are very similar to those described by Kohl,[3] and subsequently by several other investigators.

[1] Schmitz, F., *Sitzungsber. Niederrheinisch. Ges. Nat. Heilk. Bonn*, pp. 345–376 (1879).

[2] Piekarski, G., *Arch. Mikrobiol.*, **8**, 428 (1937).

[3] Kohl, F. G., *Über die Organization und Physiologie der Cyanophyceenzell und die mitotische Teilung ihres Kernes*, Jena (1903).

INEQUALITIES FOR COMPLEX LINEAR DIFFERENTIAL SYSTEMS OF THE SECOND ORDER

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The purpose of this paper is to obtain inequalities to be satisfied by solutions of systems of the form,

$$\begin{aligned} dw/dt &= \alpha w + \beta u \\ du/dt &= \gamma w + \delta u, \end{aligned} \tag{1}$$

where α, β, γ, δ are complex valued functions of the real variable t. These functions are assumed to be of class C' on some interval, $t_0 \leqq t < T$, where T may be ∞. We also restrict attention to the "general case" in which $\Delta = (\delta - \alpha)^2 + 4\beta\gamma \neq 0$ and $\gamma \neq 0$ on this interval. The inequalities obtained are the best possible ones of their type and are especially adapted to the case in which α, β, γ, δ have numerically small derivatives.

In terms of the notation,

$$\begin{aligned} \Delta &= r(t)e^{i\theta(t)} = (\delta - \alpha)^2 + 4\beta\gamma \\ R &= R(t) = |\Delta|/4\gamma\bar{\gamma} \\ D(t) &= [|w(t)|^2 + (2\bar{\gamma})^{-1}(\bar{\delta} - \bar{\alpha})w\bar{u} + (2\gamma)^{-1}(\delta - \alpha)\bar{w}u + \\ &\qquad (|2\gamma|^{-2}|\delta - \alpha|^2 + R)|u|^2]^{1/2} \\ \sigma(t) &= \alpha + \bar{\alpha} + \delta + \bar{\delta} \\ \Omega(t) &= (1 + e^{i\theta})\frac{\bar{\gamma}d}{dt}\left(\frac{\bar{\delta} - \bar{\alpha}}{2\bar{\gamma}}\right) + (1 + e^{-i\theta})\gamma\frac{d}{dt}\left(\frac{\delta - \alpha}{2\gamma}\right) + \\ &\qquad R^{-1}\left|\frac{d}{dt}\left(\frac{\delta - \alpha}{2\gamma}\right)\right|^2, \end{aligned}$$

where $\bar{w}$, $\bar{u}$, $\bar{\alpha}$, $\bar{\gamma}$, etc., are used to indicate the conjugate imaginaries of the corresponding undashed quantities, namely w, u, α, γ, etc., our fundamental inequalities can be written in the following reasonably compact form,

$$\begin{aligned} &D_0(R/R_0)^{1/4}\exp\int_{t_0}^{t}[\sigma/4 - \{(r/4)\cos^2(\theta/2) + (\dot{R}/4R)^2 + \\ &\Omega/4\}^{1/2}]\,dt \leqq D(t) \leqq D_0(R/R_0)^{1/4}\exp\int_{t_0}^{t}[\sigma/4 + \{(r/4)\cos^2(\theta/2) + \\ &\qquad (\dot{R}/4R)^2 + \Omega/4\}^{1/2}]\,dt \end{aligned} \tag{2}$$

where, of course, the functions $w(t)$ and $u(t)$ are assumed to constitute an arbitrary solution of (1). Here, as in the sequel, a dot over a letter in-

dicates differentiation with respect to t, and $D_0 = D(t_0)$ and $R_0 = R(t_0)$.

These inequalities are greatly simplified in case $\alpha = \delta$, as one sees at once from the definition of Ω. It is also easier to establish the above inequalities in this special case; moreover it is possible to obtain certain inequalities quite similar, but not identical, to (2) in the case $\alpha \neq \delta$ by means of a preliminary transformation on the dependent variables, such as

$$w^* = w + (\delta - \alpha)u/2\gamma, \quad u^* = u$$

which reduces (1) to a similar system,

$$\begin{aligned} \dot{w}^* &= \alpha^* w^* + \beta^* u^* \\ \dot{u}^* &= \gamma^* w^* + \delta^* u^*, \end{aligned}$$

in which $\delta^* = \alpha^* = 2^{-1}(\alpha + \delta)$, $\beta^* = \Delta/4\gamma + (d/dt)[(\delta - \alpha)/2\gamma]$, and $\gamma^* = \gamma$. But there are certain disadvantages in setting up inequalities in this manner. For instance, if the transformation cited above is used, we must later assume that α^*, β^*, γ^* are of class C', and hence we would have to assume that the original coefficients are of class C'' or at least that $(\delta - \alpha)/2\gamma$ is of class C''. We shall therefore establish (2) without any assumption as to a relationship between α and δ. Our proof runs as follows:

Consider the positive definite Hermitian form,

$$Q = w\bar{w} + \bar{A}w\bar{u} + A\bar{w}u + B\bar{u}u,$$

where $A = (\delta - \alpha)/2\gamma$, $B = (|\delta - \alpha|^2 + |\Delta|)/4\gamma\bar{\gamma} = A\bar{A} + R$. Assuming that w and u satisfy (1), we differentiate Q with respect to t and replace $\dot{w}$ by $\alpha w + \beta u$, $\dot{\bar{w}}$ by $\bar{\alpha}\bar{w} + \bar{\beta}\bar{u}$, $\dot{u}$ by $\gamma w + \delta u$, and $\dot{\bar{u}}$ by $\bar{\gamma}\bar{w} + \bar{\delta}\bar{u}$. We thus find that

$$\begin{aligned} \dot{Q} = {} & (\alpha + \bar{\alpha} + A\gamma + \bar{A}\bar{\gamma})w\bar{w} + (\bar{A}\alpha + \bar{\beta} + \bar{A}\bar{\delta} + B\gamma + \dot{\bar{A}})w\bar{u} + \\ & (A\bar{\alpha} + \beta + A\delta + B\bar{\gamma} + \dot{A})\bar{w}u + (\bar{A}\beta + A\bar{\beta} + B\bar{\delta} + B\delta + \dot{B})u\bar{u}. \end{aligned}$$

If we denote by Q^* the Hermitian form on the right, we have

$$\min (Q^*/Q) \leqq \dot{Q}/Q \leqq \max (Q^*/Q). \tag{3}$$

Evidently the problem of minimizing or maximizing Q^*/Q is equivalent to minimizing or maximizing Q^* for values of w and u which make $Q = 1$. This problem can be handled by the method of Lagrange, which yields the result that the minimum and maximum values of Q^*/Q are precisely the two roots of the following determinantal equation in λ:

$$\begin{vmatrix} \alpha + \bar{\alpha} + A\gamma + \bar{A}\bar{\gamma} - \lambda & \bar{A}\alpha + \bar{\beta} + \bar{A}\bar{\delta} + B\gamma + \dot{\bar{A}} - \bar{A}\lambda \\ A\bar{\alpha} + \beta + A\delta + B\bar{\gamma} + \dot{A} - A\lambda & \bar{A}\beta + A\bar{\beta} + B\bar{\delta} + B\delta + \dot{B} - B\lambda \end{vmatrix} = 0$$

When we replace A and B by their expressions in terms of α, β, γ, δ we find that

$$\alpha + \bar{\alpha} + A\gamma + \bar{A}\bar{\gamma} = (\alpha + \bar{\alpha} + \delta + \bar{\delta})/2 = \sigma/2$$
$$\bar{A}\alpha + \bar{\beta} + \bar{A}\bar{\delta} + B\gamma = (|\Delta| + \bar{\Delta} + \sigma(\bar{\delta} - \bar{\alpha}))/4\bar{\gamma}$$
$$A\bar{\alpha} + \beta + A\delta + B\bar{\gamma} = (|\Delta| + \Delta + \sigma(\delta - \alpha))/4\gamma$$
$$\bar{A}\beta + A\bar{\beta} + B\bar{\delta} + B\delta = [\sigma(|\Delta| + |\delta - \alpha|^2) + (\delta - \alpha)(|\Delta| + \Delta) + (\bar{\delta} - \bar{\alpha})(|\Delta| + \Delta)]/8\gamma\bar{\gamma}$$
$$B - A\bar{A} = |\Delta|/4\gamma\bar{\gamma} = R.$$

The above determinantal equation can be expanded with the help of these relationships and we thus arrive at the quadratic equation in λ,

$$\lambda^2 - (\sigma + R^{-1}\dot{R})\lambda + 4^{-1}(\sigma^2 - (\Delta^{1/2} + \Delta^{1/2})^2 + 2\sigma R^{-1}\dot{R}) - \Omega = 0, \quad (4)$$

where $\Delta^{1/2}$ and $\Delta^{1/2}$ are chosen so as to be conjugate imaginaries of each other. The roots of (4), both real, of course, are

$$\begin{aligned}\lambda &= 2^{-1}(\sigma + R^{-1}\dot{R}) \pm 2^{-1}[(\Delta^{1/2} + \bar{\Delta}^{1/2})^2 + R^{-2}\dot{R}^2 + 4\Omega]^{1/2} \\ &= 2^{-1}(\sigma + R^{-1}\dot{R}) \pm [r\cos^2(\theta/2) + 4^{-1}R^{-2}\dot{R}^2 + \Omega]^{1/2}.\end{aligned}$$

The $\pm$ sign is to be taken $+$ to yield the maximum for Q^*/Q and $-$ for the minimum. Both, of course, are functions of t. Our fundamental inequalities (2) follow at once upon integration of (3) and upon observing that $D = Q^{1/2}$.

We add the following two remarks:

I. In case α, β, γ and δ are constants, the roots of (4) reduce to $2^{-1}(\alpha + \delta + \bar{\alpha} + \bar{\delta}) \pm 2^{-1}(\Delta^{1/2} + \bar{\Delta}^{1/2})$, which are precisely twice the real parts of the roots of the usual characteristic equation.

$$\begin{vmatrix} \alpha - \mu & \beta \\ \gamma & \delta - \mu \end{vmatrix} = 0$$

in μ. It follows that in this case of constant coefficients there will always exist a solution for which either one of the $\leqq$ signs in (2) may be replaced by an equal sign. This remark explains the precise meaning of our claim that our inequalities are the best of their type, and it was in order to reach this result that the Hermitian form Q was chosen in the peculiar form stated. Certain other forms would lead to a similar result, but the form chosen seems to be the simplest. The algebraic background behind our choice of Q was given in a previous paper.[1]

II. As simple corollaries of our inequalities, we may read off criteria for boundedness of the functions $[w(t), u(t)]$ constituting a solution of (1). For instance, *if the larger of the two roots of* (4), *namely*

$$\lambda = 2^{-1}(\sigma + R^{-1}\dot{R}) + [r\cos^2(\theta/2) + 4^{-1}\dot{R}^2/R^2 + \Omega]^{1/2}$$

does not exceed $\dot{R}/R$ *(or even if it has only the property that*

$$\int_{t_0}^{t} (\lambda - \dot{R}/R)\, dt$$

is bounded from above), then the function $u(t)$ must be bounded. In fact $D(t) = (|w + (2\gamma)^{-1}(\delta - \alpha)u|^2 + R|u|^2)^{1/2} \leqq D_0 \exp \int_{t_0}^{t} (\lambda/2)\, dt$. Hence $|u| \leqq R^{-1/2} D_0 \exp \int_{t_0}^{t} (\lambda/2)\, dt = D_0 R_0^{-1/2} \exp \int_{t_0}^{t} (\lambda/2 - \dot{R}/2R)\, dt$, from which the italicized statement is obvious.

A slight generalization of a theorem of Leighton is to the effect that every solution of the equation $(d/dt)(Ge^{i\varphi}\, du/dt) + Fe^{i\varphi} u = 0$ is bounded, if $F > 0$, $G > 0$, and φ are real and of class C' in t, and if FG is monotonically increasing on $(t_0 \leqq t < \infty)$. This is an extremely special case of the above corollary. For, if we take $w = Ge^{i\varphi}\, \dot{u}$, the differential equation may be written in the form (1) with $\alpha = \delta = 0$, $\beta = -Fe^{i\varphi}$, $\gamma = G^{-1}e^{-i\varphi}$. Hence $\sigma = \Omega = 0$, $\Delta = -4FG^{-1}$, $\theta \equiv \pi \pmod{2\pi}$, $R = FG$. Remembering that this R is monotonically increasing, we can write $(\dot{R}^2)^{1/2} = +\dot{R}$. A simple calculation, now shows that $\lambda = \dot{R}/R$, so that the above criterion is satisfied.

[1] Lewis, D. C., "Differential Equations Referred to a Variable Metric," *Am. Jour. Math.*, **73**, 48–58 (1951). Especially Theorem 3, pp. 56 and 57.

[2] Leighton, W., "Bound for the Solutions of a Second-Order Linear Differential Equation," PROC. NATL. ACAD. SCI., **35**, 190–191 (1949).

A TOPOLOGICAL CHARACTERIZATION OF THE LENGTH OF PATHS.

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Let T be a metrizable topological space. We speak of a universal functional of paths if with every path $\mathcal{P}$ in T a (finite or infinite) number $\lambda\mathcal{P}$ is associated. For any particular metrization of T, the corresponding length of paths is an example of a non-negative universal functional. To different metrizations of T correspond, in general, different lengths. *How are these lengths characterized among the non-negative universal functionals of paths?* In other words, what properties of a functional λ are necessary and sufficient in order that there exist a metrization of T such that, for every path $\mathcal{P}$, the corresponding length is equal to $\lambda\mathcal{P}$? We widen the scope of the problem by admitting metrizations of T for which the distance is non-symmetric.

1. What Is a Path? In the classical theory, a path is obtained from a motion by a process of abstraction. A motion M in T is a continuous association of a point $p_M(t)$ of T with each moment t of a closed interval $[a, b]$. Abstracting from kinematical aspects of the motion, Fréchet called the motion N: $q_N(u)$ $(c \leq u \leq d)$ *equivalent* with M if there exists a quasi-

similarity between the intervals $[a, b]$ and $[c, d]$ such that $p_M(t) = q_N(u)$ for any two related moments t and u. Here, by a quasi-similarity we mean a set of pairs (t, u) of related moments such that (1) each $t \in [a, b]$ as well as each $u \in [c, d]$ belongs to at least one pair; (2) if (t, u) and (t', u') are two pairs of related moments, then $(t' - t) \cdot (u' - u) \geq 0$.

The *path of* M is the set of all motions which are equivalent to M. *Path* is the path of some motion. One readily defines convergence of paths. The terms initial point and terminal point of a path are self-explanatory. If the terminal point of $\mathfrak{P}$ coincides with the initial point of $\mathfrak{Q}$, then we call $\mathfrak{Q}$ *consecutive* to $\mathfrak{P}$, and we denote by $\mathfrak{P} + \mathfrak{Q}$ the combined path from the initial point of $\mathfrak{P}$ to the terminal point of $\mathfrak{Q}$.

In an alternate theory, we have more recently[1] defined the path of M by adding geometric specifications to the trajectory of M, i.e., the set of all positions $p_M(t)$ $(a \leq t \leq b)$. This trajectory is the same for any equivalent motion and hence may be called the trajectory of the path. But also non-equivalent motions and different paths may have the same trajectory. While thus the set of points of M is insufficient to characterize the path of M, we may define the latter as *the set of all polygons of* M, that is, the set of finite sequences of points $\{p_M(t_1), p_M(t_2), \ldots, p_M(t_n)\}$ for all ordered sets $\{t_1, t_2, \ldots, t_n\}$ for which $a \leq t_1 < t_2 < \ldots < t_n \leq b$. *Path* is the set of all polygons of some motion.

The sets of polygons which are paths, can be characterized by intrinsic properties without any reference to motions or particular parametrizations. If, for the sake of a simpler formulation, we endow T with a particular metric, a path in T is a set $\mathfrak{P}$ of polygons (finite sequences of points of T) with the following properties:

1. $\mathfrak{P}$ *contains polygons whose norms relative to* $\mathfrak{P}$ *are arbitrarily small.* By the norm of $P = \{p_1, \ldots, p_n\}$ relative to $\mathfrak{P}$ we mean the least upper bound of the diameters of all polygons $\in \mathfrak{P}$ from any p_i to the consecutive p_{i+1} $(i = 1, \ldots, n - 1)$, the initial and the terminal point of $\mathfrak{P}$ being, if necessary, adjoined to P.

2. $\mathfrak{P}$ *contains for every two of its polygons a common refinement.* The polygon P' is called a refinement of P if the sequence P is a subsequence of the sequence P'.

3. $\mathfrak{P}$ *is saturated with regard to properties 1 and 2* in the sense that $\mathfrak{P}$ is not a proper subset of a set of polygons $\mathfrak{P}'$ containing a common refinement for every two of its polygons and such that $\mathfrak{P}$ contains polygons of arbitrarily small norms relative to $\mathfrak{P}'$ (even though $\mathfrak{P}'$ itself may contain polygons of arbitrarily small norms relative to $\mathfrak{P}'$).

II. What Is Length? If T is endowed with a particular (not necessarily symmetric) metric, the length $l\mathfrak{P}$ of the path $\mathfrak{P}$ is defined as the least upper bound of the lengths of all polygons of $\mathfrak{P}$, the length of $P = \{p_1, \ldots, p_n\}$ being $\sum d(p_{i-1}, p_i)$. For any metric, the corresponding length of paths is

(*A*) *additive:* if $\mathfrak{Q}$ is consecutive to $\mathfrak{P}$, then $l(\mathfrak{P} + \mathfrak{Q}) = l\mathfrak{P} + l\mathfrak{Q}$;
(*B*) *lower semi-continuous:* if $\lim \mathfrak{P}_n = \mathfrak{P}$, then $\liminf l\mathfrak{P}_n \geq l\mathfrak{P}$;
(*C*) *regular:* if, for each n, $\mathfrak{P}_n$ is a path from p to p_n and $\lim l\mathfrak{P}_n = 0$, then $\lim p_n = p$.

The properties A, B, C characterize the lengths among the non-negative universal functionals λ *of paths contained in a compact metrizable space T.* Suppose T and λ are given. First we assume T to be λ-*connected* (i.e., to contain, for any two points p, q, a path $\mathfrak{P}$ for which $\lambda\mathfrak{P} < \infty$) and *locally* λ-*connected* (i.e., to contain, if $\lim p_n = p$, a path $\mathfrak{P}_n$ from p to p_n such that $\lim \lambda\mathfrak{P}_n = 0$). If λ has the properties A, B, C, then λ is the length relative to a metric by virtue of which T is *convex* in the sense that, for every two points p and q, T contains at least one path from p to q whose length is $d(p, q)$. We obtain the corresponding distance by setting

$$d(p, q) = g.l.b.\lambda\mathfrak{P} \text{ for all paths } \mathfrak{P} \text{ from } p \text{ to } q.$$

It can be shown[2] that $d(p, p) = 0$; $d(p, q) > 0$ if $p \neq q$; $d(p, q) + d(q, r) \geq d(p, r)$; and $\lim d(p_n, p) = 0$ if and only $\lim p_n = p$. It remains to be proved that $l\mathfrak{P} = \lambda\mathfrak{P}$. For any $\epsilon > 0$, let $P = \{p_0, \ldots, p_n\}$ be a polygon of $\mathfrak{P}$ such that $\sum d(p_{i-1}, p_i) > l\mathfrak{P} - \epsilon$. If $\mathfrak{P}_1, \ldots, \mathfrak{P}_n$ denote the segments into which P divides $\mathfrak{P}$, then $\lambda\mathfrak{P} = \sum\lambda\mathfrak{P}_i \geq \sum d(p_{i-1}, p_i) > l\mathfrak{P} - \epsilon$. Conversely, there exist paths $\mathfrak{Q}_i$ from p_{i-1} to p_i such that $\lambda\mathfrak{Q}_i < d(p_{i-1}, p_i) + \frac{\epsilon}{n}$. Setting $\mathfrak{Q} = \mathfrak{Q}_1 + \ldots + \mathfrak{Q}_n$ we have $\lambda\mathfrak{Q} < \Sigma d(p_{i-1}, p_i) + \epsilon < l\mathfrak{P} + \epsilon$. Thus in each neighborhood of $\mathfrak{P}$ there exist paths $\mathfrak{Q}$ for which $\lambda\mathfrak{Q} < l\mathfrak{P} + \epsilon$. Since λ is lower semicontinuous, $\lambda\mathfrak{P} \leq l\mathfrak{P}$.

III. Generalizations and Problems. Carathéodory's theorem concerning the compactness of the set of all curves of uniformly bounded length can be generalized as follows.[3] Let λ be any non-negative universal functional of paths contained in a compact metrizable space T. If λ has the properties A, B, C, then, for every $L < \infty$, the set of all paths $\mathfrak{P}$ for which $\lambda\mathfrak{P} \leq L$, is compact. Using this generalization we prove the following theorem. Let λ be a non-negative universal functional of the paths contained in a compact metrizable space T. *In order that* λ *be the length in a metroid M which* (1) *contains a path of length* $d(p, q)$ *from p to q whenever* $d(p, q) < \infty$, (2) *can be mapped on T in a one-to-one continuous way and so that* $l\mathfrak{P} = \lambda\mathfrak{P}$ *for every path, it is necessary and sufficient that* λ *have the properties A, B, C.* Here, by a metroid we mean a set in which every two points have a finite or infinite distance such that $d(p, p) = 0$; $d(p, q) > 0$ if $p \neq q$; $d(p, q) + d(q, r) \geq d(p, r)$. *If T is* λ-*connected, then a metric M can be chosen* (in which all distances are finite); *if T is locally* λ-*connected, then M can be mapped on T topologically.*

Topological characterizations of length whenever T is homeomorphic with a complete metric space would be desirable; similarly, characteriza-

tions of length in spaces where the distances satisfy only the condition that $\lim p_n = p$ implies $\lim d(p_n, p) = 0$ (without necessarily being non-negative, triangular, etc.). In such spaces, length is not necessarily a universal functional.[4] Most interesting would be extensions to the area of surfaces in the sense of continuous images of a disk. Such surfaces might be defined as sets of polyhedra.

[1] "Définition intrinsèque de la notion de chemin" *C. R. Acad. Paris*, **221**, 739 (1945).

[2] "Untersuchungen ueber allgemeine Metrik, I," *Mathem. Annalen*, **100**, 96 sq. (1928).

[3] For a slightly weaker generalization cf. "Metric Methods in Calculus of Variations," PROC. NATL. ACAD. SCI., **23**, 247 (1937), and "A Theory of Length and Its Applications to the Calculus of Variations," *Ibid.*, **25**, 476 (1939).

[4] "What Paths Have Length?" *Fund. Math.*, **36**, 109-118 (1949).

THE ELEMENT OF VOLUME OF THE ROTATION GROUP

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The element of volume of the n-dimensional rotation group may be written as the product of two factors one of which, the class factor, is well known. The other factor, which involves those parameters of the group which vary over a given class (and which we shall term the in-class factor) has not, to our knowledge, been heretofore determined. In a previous note[1] we gave the element of volume of the n-dimensional rotation group in terms of parameters which may be termed Eulerian since they are an extension to a general n of a modification of the Eulerian angles ϕ, θ, ψ which serve as parameters for the 3-dimensional rotation group and which are of fundamental importance in rigid dynamics. Thus any 3×3 rotation matrix R_3 may be written as the product of three plane rotation matrices as follows:

$$R_3 = R_{12}(\phi)R_{31}(\theta)R_{12}(\psi) \qquad \text{where } R_{12}(\phi) = \begin{pmatrix} \cos\phi & -\sin\phi & 0 \\ \sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{pmatrix} \text{etc.},$$

and the element of volume of the 3-dimensional rotation group, in terms of the parameters ϕ, θ, ψ, is $\sin\theta\, d\phi\, d\theta\, d\psi$ (or the product of this by any positive constant). The parameter θ is a latitude, or half-turn, angle which ranges over the interval 0 to π while the parameters ϕ and ψ are longitude, or whole-turn, angles which range over the interval $-\pi$ to π. These Eulerian parameters suffer from the defect that they do not provide a

separation of the class parameter (which must be a function of the angle α, $0 \leqslant \alpha \leqslant \pi$, of the rotation R_3) from the in-class parameters, all the parameters ϕ, θ, ψ varying when α is given. We provide in this note a system of parameters for the n-dimensional rotation group in which the class parameters are separated from the in-class parameters and we give the complete element of volume for the cases $n = 3, 4, 5, 6, 7$. We show also how to determine the element of volume of the $(2m + 1)$-dimensional rotation group when the element of volume of the $2m$-dimensional rotation group is known.

$n = 3$. Any 3×3 rotation matrix R_3 may be written as the product of plane rotation matrices as follows:

$$R_3 = A^* R_{12}(\alpha) A; \quad A = R_{23}(\theta') R_{12}(\phi').$$

Here A^* is the reciprocal of A; α, the angle of R_3, is the class parameter and is a half-turn angle; θ' and ϕ' are the in-class parameters of which θ' is a half-turn, and ϕ' a whole-turn, angle. The axis of R_3 is furnished by the unit vector

$$\mathbf{e}_1 = (\sin\theta' \sin\phi')\mathbf{i} + (\sin\theta' \cos\phi')\mathbf{j} + (\cos\theta')\mathbf{k},$$

where $\mathbf{i}$, $\mathbf{j}$, $\mathbf{k}$ are the unit base vectors of the moving reference frame and the intersection of the plane of the rotation R_3 with the moving equatorial plane is furnished by the unit vector $\mathbf{e}_2 = (\cos\phi')\,\mathbf{i} - (\sin\phi')\mathbf{j}$. The angular velocity of the moving reference frame is

$$\boldsymbol{\omega} = \alpha_t \mathbf{e}_1 + \{(1 - \cos\alpha)\theta_t' + \sin\alpha \sin\theta' \phi_t'\}\mathbf{e}_2 + \{(\sin\alpha)\theta_t' - \sin\theta'(1 - \cos\alpha)\phi_t'\}(\mathbf{e}_1 \times \mathbf{e}_2),$$

where the subscript t denotes time differentiation. The class factor of the element of volume is $(1 - \cos\alpha)\, d\alpha$ and the in-class factor is $\sin\theta'\, d\theta'\, d\phi'$ so that the complete element of volume is $dV_3 = (1 - \cos\alpha) \sin\theta'\, d\alpha\, d\theta'\, d\phi'$. This is the product of the element of volume $dV_2 = d\alpha$ of the 2-dimensional rotation group by $(1 - \cos\alpha) \sin\theta'\, d\theta'\, d\phi'$.

$n = 4$. Any 4×4 rotation matrix R_4 may be written as the product of plane rotation matrices as follows:

$$R_4 = A^* R_{24}^*(\beta) R_{12}(\alpha_1) R_{34}(\alpha_2) R_{24}(\beta) A; \quad A = R_{12}(\phi_1) R_{23}(\theta) R_{12}(\phi_2).$$

α_1 and α_2, the two angles of R_4, are the class parameters and are half-turn angles; β is a quarter-turn angle $\left(\text{i.e., it ranges over the interval 0 to } \frac{\pi}{2}\right)$ and ϕ_1, θ, ϕ_2, which are related in a simple manner to the Eulerian parameters of the 3-dimensional rotation group, are such that θ is a half-turn angle while ϕ_1 and ϕ_2 are whole-turn angles. The class factor of the element of volume is $\{1 - \cos(\alpha_1 + \alpha_2)\}\{1 - \cos(\alpha_1 - \alpha_2)\}\, d\alpha_1\, d\alpha_2$ while the in-class

factor is $\sin 2\beta \sin\theta\, d\beta\, d\phi_1\, d\theta\, d\phi_2$. Thus the complete element of volume is

$$dV_4 = \{1 - \cos(\alpha_1 + \alpha_2)\}\{1 - \cos(\alpha_1 - \alpha_2)\} \sin 2\beta \sin\theta\, d\alpha_1\, d\alpha_2\, d\beta\, d\phi_1\, d\theta\, d\phi_2$$

(or the product of this by any positive constant). NOTE: The factorization $R_{12}(\phi_1)R_{23}(\theta)\,R_{12}(\phi_2)$ of any 3×3 rotation matrix is more convenient for our purpose than the Eulerian factorization $R_{12}(\phi)R_{31}(\theta)R_{12}(\psi)$ and $\phi_1 = -\left(\phi + \frac{\pi}{2}\right)$, $\phi_2 = \psi - \frac{\pi}{2}$. We shall term ϕ_1, θ, ϕ_2 the modified Eulerian parameters.

$n = 5$. The 5-dimensional rotation group has the same number of class parameters, namely 2, as the 4-dimensional rotation group (just as the 3-dimensional rotation group has the same number of class parameters, namely 1, as the 2-dimensional rotation group). It has four additional in-class parameters three of which, which we denote by θ_1', θ_2', θ_3', are half-turn angles and one of which, which we denote by ϕ', is a whole-turn angle. Any 5×5 rotation matrix R_5 may be factored into plane rotation matrices as follows:

$$R_5 = B^* R_4 B; \qquad B = R_{45}(\theta_1')R_{34}(\theta_2')R_{23}(\theta_3')R_{12}(\phi'),$$

where $R_4 = A^* R_{24}^*(\beta)R_{12}(\alpha_1)R_{34}(\alpha_2)R_{24}(\beta)A$ is the factorization which furnished the parameters of the 4-dimensional rotation group (it being understood that each factor is extended, so as to be a 5×5, rather than a 4×4, rotation matrix, by the addition of a last column and a last row all of whose elements are zero save the common diagonal element which is 1). The class factor of dV_5 is the product of the class factor of dV_4 by (1-cos α_1) (1-cos α_2), just as the class factor of dV_3 is the product of the class factor of dV_2 (i.e., dV_2 itself) by (1-cos α). The in-class factor of dV_5 is the product of the in-class factor of dV_4 by $\sin^3\theta_1' \sin^2\theta_2' \sin\theta_3'\, d\theta_1'\, d\theta_2'\, d\theta_3'\, d\phi'$, just as the in-class factor of dV_3 is the product of the in-class factor of dV_2 (i.e., 1) by $\sin\theta'\, d\theta'\, d\phi'$. Thus the complete element of volume of the 5-dimensional rotation group is

$$dV_5 = (1 - \cos\alpha_1)(1 - \cos\alpha_2)\{1 - \cos(\alpha_1 + \alpha_2)\}\{1 - \cos(\alpha_1 - \alpha_2)\} \sin 2\beta \sin\theta \sin^3\theta_1' \sin^2\theta_2' \sin\theta_3'\, d\alpha_1\, d\alpha_2\, d\beta\, d\theta\, d\varphi_1\, d\varphi_2\, d\theta_1'\, d\theta_2'\, d\theta_3'\, d\varphi'$$

(or the product of this by any positive constant).

$n = 6$. The 6-dimensional rotation group has 3 class parameters α_1, α_2, α_3, the angles of the rotation R_6 (which are all half-turn angles) and it has 12 in-class parameters of which two, β_1 and β_2, are quarter-turn angles and the remaining ten may be taken to be the modified Eulerian parameters of the 5-dimensional rotation group. Thus every 6×6 rotation matrix R_6 may be written as the product of plane rotation matrices as follows

$$R_6 = A^*R_{46}^*(\beta_2)\ R_{24}^*(\beta_1)\ R_{12}(\alpha_1)R_{34}(\alpha_2)R_{56}(\alpha_3)R_{24}(\beta_1)R_{46}(\beta_2)A$$

where $A = R_{12}(\phi_1)R_{23}(\theta_1)R_{34}(\theta_2)R_{45}(\theta_3)R_{12}(\phi_2)R_{23}(\theta_4)R_{34}(\theta_5)R_{12}(\phi_3)R_{23}(\theta_6)$-$R_{12}(\phi_4)$. The coefficient of $d\alpha_1\, d\alpha_2\, d\alpha_3$ in the class factor of dV_6 is the product $\prod_{p<q} \{1 - \cos(\alpha_p + \alpha_q)\}\{1 - \cos(\alpha_p - \alpha_q)\}$ and the in-class factor of dV_6 is

$$\sin 2\beta_1(1 - \cos 2\beta_2) \sin 2\beta_2 \sin\theta_1 \sin^2\theta_2 \sin\theta_3 \sin^3\theta_4 \sin^2\theta_5 \sin\theta_6$$
$$d\beta_1\, d\beta_2\, d\theta_1 \ldots d\theta_6\, d\varphi_1 \ldots d\varphi_4.$$

$n = 7$. The 7-dimensional rotation group has the same number of class parameters, namely 3, as the 6-dimensional rotation group and the class factor of dV_7 is the product of the class factor of dV_6 by $(1 - \cos\alpha_1)$ $(1 - \cos\alpha_2)$ $(1 - \cos\alpha_3)$. It has six more in-class parameters of which 5, which we denote by $\theta_1', \theta_2', \ldots, \theta_5'$, are half-turn angles while the remaining one, which we denote by ϕ', is a whole-turn angle. Any 7×7 rotation matrix may be factored into plane rotation matrices as follows

$$R_7 = B^*R_6B; \qquad B = R_{67}(\theta_1')R_{56}(\theta_2')R_{45}(\theta_3')R_{34}(\theta_4')R_{23}(\theta_5')R_{12}(\phi')$$

where $R_6 = A^*R_{46}^*(\beta_2)R_{24}^*(\beta_1)R_{12}(\alpha_1)R_{34}(\alpha_2)R_{56}(\alpha_3)R_{24}(\beta_1)R_{46}(\beta_2)A$ is the factorization which furnished the parameters of the 6-dimensional rotation group (it being understood, as before, that each factor is extended, so as to be a 7×7, rather than a 6×6, rotation matrix, by the addition of a last column and a last row all of whose elements are zero save the common diagonal element which is 1). The in-class factor of dV_7 is the product of the in-class factor of dV_6 by $\sin^5\theta_1' \sin^4\theta_2' \sin^3\theta_3' \sin^2\theta_4' \sin\theta_5'\, d\theta_1' \ldots d\theta_5'\, d\phi'$.

The $2m$-dimensional rotation group has m class parameters, $\alpha_1, \ldots, \alpha_m$, the angles of R_{2m}, each of which is a half-turn angle. It has $2m(m-1)$ in-class parameters of which $m - 1$, $\beta_1, \ldots, \beta_{m-1}$, are quarter-turn angles while the remaining $(2m - 1)$ $(m - 1)$ may be taken to be the modified Eulerian parameters of the $(2m - 1)$-dimensional rotation group. An arbitrary $2m \times 2m$ rotation matrix may be factored into plane rotation matrices as follows:

$$R_{2m} = A^*C^*\ R_{12}(\alpha_1)R_{34}(\alpha_2) \ldots R_{2m-1,\,2m}(\alpha_m)CA$$

where $C = R_{24}(\beta_1)R_{46}(\beta_2) \ldots R_{2m-2,\,2m}(\beta_{m-1})$ and A is the modified Euler factorization of a typical element of the $(2m - 1)$-dimensional rotation group

$$A = R_{12}(\phi_1)R_{23}(\theta_1) \ldots R_{12}(\phi_{2m-2})$$

it being understood that each of the factors is extended so as to be a $2m \times 2m$, rather than a $(2m - 1) \times (2m - 1)$, rotation matrix. The coefficient

of $d\alpha_1 \ldots d\alpha_m$ in the class factor of dV_{2m} is the product $\prod_{p<q} (1 - \cos(\alpha_p + \alpha_q))(1 - \cos(\alpha_p - \alpha_q))$. When we pass from the $2m$-dimensional rotation group to the $(2m + 1)$-dimensional rotation group there is no change in the number, namely m, of class parameters and the class factor of dV_{2m+1} is the product of the class factor of dV_{2m} by $(1 - \cos \alpha_1) \ldots (1 - \cos \alpha_m)$. There are $2m$ additional in-class parameters of which $2m - 1$, which we denote by $\theta_1', \ldots, \theta_{2m-1}'$ are half-turn angles, the remaining parameter, which we denote by ϕ', being a whole-turn angle. The in-class factor of dV_{2m+1} is the product of the in-class factor of dV_{2m} by $\sin^{2m-1}\theta_1' \sin^{2m-2}\theta_2' \ldots \sin\theta_{2m-1}' d\theta_1' \ldots d\theta_{2m-1}' d\phi'$.

NOTE ADDED IN PROOF: The in-class factor, of dV_{2n} is the product of $d\beta_1 \ldots d\beta_{n-1}\, d\phi_1 \ldots d\phi_{2n-2} d\theta_1 \ldots d\theta_{(n-1)(2n-3)}$ by two factors one of which involves the parameters β and the other the parameters θ. The factor involving the parameters β is $\sin\beta_1 \cos\beta_1 \sin^3\beta_2 \cos\beta_2 \sin^5\beta_3 \cos\beta_2 \ldots \sin^{2n-3}\beta_{n-1} \cos\beta_{n-1}$ and the factor involving the parameters θ is $(\sin\theta_1)(\sin^2\theta_2 \sin\theta_3)(\sin^3\theta_4 \sin^2\theta_5 \sin\theta_6) \ldots$ there being $2n - 3$ factors of which the last is $\sin^{2n-3}\theta_{2n^2-7n+7} \ldots \sin\theta_{(n-1)(2n-3)}$.

[1] Murnaghan, F. D., these PROCEEDINGS, **36**, 670–672 (1950).

*EFFECT OF BILATERAL ADRENALECTOMY ON CERTAIN HUMAN TUMORS**

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In 1941 it was shown[1] that elimination or inactivation of androgenic hormones of the C_{19} series (by orchiectomy or competitive inhibition with estrogenic substances respectively) caused regression of certain widely disseminated neoplasms. These happened to be cancers of man, specifically of the prostatic glands. The new principle involved was that activity of certain cancers is dependent upon the function of a remote tissue (in this case, the testis) which is operating not at a heightened level of activity but at a normal, or more usually, at a subnormal rate of activity. By depriving the organism of significant quantities of C_{19} androgens some cancers became controlled by the use of forces resident in the organism.

Another locus where steroid hormones are formed to an important degree is the adrenal cortex. We shall present proof that the adrenal glands in certain cases of cancer activate the neoplasm since their removal deactivates the lesion.

It is known that excision of the adrenal glands with subsequent maintenance of the organism on sodium chloride has 2 opposite effects on tumors. Murphy and Sturm[2] showed that adrenalectomy increases the activity of lymphoid tumors in the rat; Ingle and Baker[3] with precise methods proved that adrenalectomy retards the growth of transplantable tumors, also in rats. A decrease in the size of established tumors has not been described.

Bilateral simultaneous adrenalectomy has been carried out by us on 28 human cases with 2 deaths; there have been no fatalities in the last 24 consecutive cases. Cortisone acetate has made this possible. Following the rather large replacement doses of cortisone acetate and DOCA required to insure the prevention of adrenal insufficiency, the patient within a week is placed on a hormonal substitution program which in most cases proves to be the future maintenance regimen. Criteria for adequate substitution were the prevention of any sign or symptom of adrenal insufficiency. Maintenance of electrolyte and carbohydrate balance and of adequate blood pressure without orthostatic hypotension were the best indicators. In most cases 25 mg. of cortisone acetate by mouth twice daily and the oral supplement to the diet of 3 g. of sodium chloride gave excellent results.

When maintained adequately, adrenalectomized patients have a healthy appearance, are not incapacitated and are able to engage in all of their usual activities. They withstand well the common ordeals of life; under severe emergencies such as extreme heat or severe infections, signs of adrenal insufficiency develop occasionally but are easily eliminated by increasing the amount of cortisone and DOCA. Addisonian pigmentation does not occur.

There were 18 patients in this series, subdivided in 3 groups; namely, prostatic, mammary and miscellaneous cancers. All of the neoplasms were far advanced and had metastasized widely. The methods for the adrenalectomy and the subsequent management have been described.[4] The period of post-operative observation has been 4 to 9 months.

Adrenalectomy for Prostatic Cancer.—There were 7 patients with far advanced cancer of the prostate; all had been treated by orchiectomy and had had a relapse after having had a remission. There was 1 death in the series leaving 6 effective cases. There was a remission of magnitude in 3 of these cases; 2 others showed appreciable improvement and in 1 case there was no beneficial effect. Among the evidences of regression of the lesions were: gain in body weight, increase of plasma proteins, decreased least percentage of thermocoagulable protein of serum, decrease of elevated acid phosphatase in serum, increased hemoglobin and red cell content of the blood, shrinkage of size of the cancers. Three patients in this series had been bedridden and were subsequently able to return to work. Perhaps

the most striking observation has been the immediate and persistent relief of crippling bone pain.

Adrenalectomy for Advanced Mammary Cancer.—There were 7 patients in this series with mammary cancer; one died post-operatively. The advance of the disease seemed to be unimpeded in 2. However, 3 of the 6 effective patients had a significant remission of the disease and 1 patient was slightly improved.

In 2 patients both having pulmonary metastases there was an increase in quantity of the serum proteins, gain in weight, relief of symptoms and some regression of the lung lesions. A woman with massive hemothorax, the fluid containing cancer cells, did not require thoracentesis after adrenalectomy. A man with cerebral and pulmonary metastasis had regression of both lesions after adrenalectomy.

In 1 patient there was extensive recurrence of cancer in a post-mastectomy scar and a large tumor in the opposite breast. Here adrenalectomy caused regression of both lesions.

Adrenalectomy for Miscellaneous Cancers.—There were 4 extensive neoplasms in this category—a squamous carcinoma, chorionic epithelioma, melanosarcoma, and pulmonary carcinoma. None of these patients had any regression of the lesions after adrenalectomy.

It is of interest that adrenalectomy can induce regression of certain malignant tumors and yet is totally unable to influence favorably other neoplasms having the same site of origin. This is further evidence, as we have shown before, that the functional characteristics of cancers vary widely. A considerable number of cancers retain some or many functional characteristics of normal cells.

In this small series of cases only prostatic and mammary carcinoma and not all of them underwent regression. Now it is established that sex hormones can induce regression or increase activity of certain cancers of breast and prostate. Since regressions after adrenalectomy occur while the patient is maintained on cortisone acetate, it may be inferred that steroids of this type are not responsible for continuing functional activity of the neoplasm. It is most reasonable to assume that the regressions are due at least in part to elimination of critical amounts of sex hormones by adrenalectomy. It must be pointed out however that adrenalectomy seems to have a non-specific effect in retarding growth of several transplantable tumors of rodents and this non-specific effect may be partially operative in prostatic and mammary tumors. Since there were no regressions in the class of "miscellaneous" neoplasms after adrenalectomy, it may be inferred that the non-specific effect is of minor magnitude.

Conclusion.—The activity of certain neoplasms is dependent upon function of the adrenal glands and these tumors regress somewhat after adrenalectomy with maintenance of the host on cortisone acetate.

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CAUCHY'S FORMULA ON KÄHLER MANIFOLDS

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1. A Cauchy's formula for analytic differential forms on a complex manifold expresses the value of the form at a point of a given subdomain in terms of an integral over the boundary of that domain, and it should satisfy the following two requirements: (i) the integrand should involve only the values of the form on the boundary; (ii) the kernel or kernels appearing is the integrand should be independent of the given subdomain.

Our purpose here is to show that any proper compact subdomain B_0 of an arbitrary Kähler manifold possesses a kernel in terms of which complex analytic forms on B_0 have a Cauchy representation relative to any subdomain of B_0.

2. In reference 3 a calculus of complex operators on Kähler manifolds was developed, and the operators were applied to obtain results concerning boundary values of complex forms on Kähler manifolds. The same operators* have been introduced independently by Hodge.[5] Here we redefine briefly these operators, and we follow reference 3.

On a complex manifold there is a "quadrantal versor" which is a real tensor h_i^j whose components in the complex coordinates $z^1, z^2, \ldots, z^k$ are

$$h_i^j(z) = \begin{cases} \sqrt{-1}, & 1 \leq i = j \leq k \\ -\sqrt{-1}, & k+1 \leq i = j \leq 2k. \\ 0 & i \neq j \end{cases}$$

The complex manifold is a Kähler manifold if it carries a Kähler metric which is a Riemannian metric g_{ij} satisfying the following two conditions:

$$(a)\ g_{ij} = g_{pq}h_i^p h_j^q;$$

$$(b)\ D_p(h_i^j\phi_j) = h_i^j D_p\phi_j.$$

Here D_p denotes covariant differentiation with respect to the index p. Condition (a) states that the vectors ϕ_i and $(h\phi)_i = h_i^j\phi_j$ have the same length while (b) states that the operators h and D commute: $Dh = hD$ Let

$$h_{ij} = g_{jp}h_i^p.$$

Then (a) implies that h_{ij} is skew-symmetric and satisfies

$$h_{ij} = h_{pq}h_i^p h_j^q$$

while (b) implies that the 2-form ω with components h_{ij} is both closed and co-closed—that is $d\omega = \delta\omega = 0$ or, in other words, ω defines a harmonic field.

Following reference 2 let

$$\underset{1,0}{\Pi}{}_i^j = \frac{1}{2}\left(g_i^j - \sqrt{-1}\, h_i^j\right),\ \underset{0,1}{\Pi}{}_i^j = \overline{\underset{1,0}{\Pi}{}_i^j} = \frac{1}{2}\left(g_i^j + \sqrt{-1}\, h_i^j\right),$$

$$\underset{\rho,\sigma}{\Pi}{}_{i_1\ldots i_\rho}^{j_1\ldots j_\rho} = g_{i_1\ldots i_\rho}{}^{m_1\ldots m_\rho n_1\ldots n_\sigma}\underset{1,0}{\Pi}{}_{m_1}^{r_1}\cdots\underset{1,0}{\Pi}{}_{m_\rho}^{r_\rho}\underset{0,1}{\Pi}{}_{n_1}^{s_1}\cdots$$
$$\underset{0,1}{\Pi}{}_{n_\sigma}^{s_\sigma}\, g_{(r_1\ldots r_\rho)(s_1\ldots s_\sigma)}{}^{j_1\ldots j_\rho}$$

Here indices enclosed in parentheses are ordered according to increasing magnitude. The requirement

$$\varphi = \underset{\rho,\sigma}{\Pi}\varphi$$

is then precisely equivalent to the statement in the language of Hodge that ϕ is of type (ρ, σ).

As in reference 3 we set

$$\partial\varphi = \underset{\rho+1,\sigma}{\Pi}\, d\, \underset{\rho,\sigma}{\Pi},\ \vartheta\varphi = \underset{\rho,\sigma-1}{\Pi}\, \delta\, \underset{\rho,\sigma}{\Pi},$$

$$\bar\partial\varphi = \underset{\rho,\sigma+1}{\Pi}\, d\, \underset{\rho,\sigma}{\Pi},\quad \bar\vartheta\varphi = \underset{\rho-1,\sigma}{\Pi}\, \delta\, \underset{\rho,\sigma}{\Pi},$$

$$\square = \bar\vartheta\partial + \partial\bar\vartheta,\ \square = \bar\vartheta\bar\partial + \bar\partial\bar\vartheta.$$

We have the following identities:

$$\partial^2 = \bar\vartheta^2 = 0,\ \partial\bar\partial + \bar\partial\partial = 0,\ \bar\vartheta\vartheta + \vartheta\bar\vartheta = 0,$$
$$\bar\partial\bar\vartheta + \bar\vartheta\bar\partial = \partial\vartheta + \vartheta\partial = 0 \quad (2.1)$$

$$\Delta\,\underset{\rho,\sigma}{\Pi} = \underset{\rho,\sigma}{\Pi}\,\Delta = \square + \bar\square,\ \Delta = \delta d + d\delta, \quad (2.2)$$

$$\square = \overline{\square}. \tag{2.3}$$

These identities are contained in reference 3 with the exception of (2.3) which was pointed out by Hodge; we remark that (2.3) follows almost at once from the identity (2.26) of reference 3.

3. A fundamental singularity $\gamma_p(z, \zeta)$ for the operator Δ on a subdomain B of the Kähler manifold is one satisfying the following two conditions:

(*a*) $\Delta_z \gamma_p(z, \zeta) = 0, z \neq \zeta$,

(*b*) *As z approaches* ζ, $\gamma_p(z, \zeta)$ *behaves asymptotically like*

$$\frac{1}{2(k-1)s_k r^{2(k-1)}} g_{(i_1 \ldots i_p),(j_1 \ldots j_p)}\, dz^{i_1}\Lambda \ldots \Lambda dz^{i_p} \cdot d\zeta^{j_1}\Lambda \ldots \Lambda d\zeta^{j_p}$$

where s_k *is the volume of the unit* $2k$*-sphere and* $r = r(z, \zeta)$ *is the geodesic distance of the points* z *and* ζ.

It is proved in reference 3 that a fundamental singularity for Δ exists on a domain B with sufficiently regular boundary if and only if the following uniqueness condition is satisfied:

(*U*) *Any form* ϕ *satisfying* $\Delta\phi = 0$ *in* B *with*

$$|\phi| = \sqrt{\phi_{(i_1 \ldots i_p)}\, \bar{\phi}^{(i_1 \ldots i_p)}}$$

tending to zero as the distance from the boundary approaches zero, vanishes identically.

A fundamental singularity $\theta_p(z, \bar{\zeta})$ for the operator $\square$ on a subdomain B satisfies the conditions

(a') $\square_z \theta_p(z, \bar{\zeta}) = 0, z \neq \zeta$,

(b') *As z approaches* ζ, $\theta_p(z, \bar{\zeta})$ *behaves asymptotically like*

$$\underset{p,\sigma}{\Pi_z}\; z\; \underset{\sigma,\rho}{\Pi_\zeta}\; \gamma_p(z, \zeta).$$

If B possesses a singularity γ_p, then

$$\theta_p(z, \bar{\zeta}) = \underset{p,\sigma}{\Pi_z}\; \underset{\sigma,\rho}{\Pi_\zeta}\; \gamma_p(z, \zeta)$$

is clearly a singularity for $\square$ on B.

On the other hand, it is proved in reference 3 that θ_p exists on a domain B if and only if (U) is satisfied for the special forms $\varphi = \underset{p,\sigma}{\Pi}\, \varphi$.

We observe that if

$$\varphi = \underset{p,\sigma}{\Pi}\, \varphi,\ \square\varphi = \frac{1}{2}\Delta\varphi = 0 \text{ in } B, \qquad |\varphi| = 0 \text{ on the boundary}, \tag{3.1}$$

then by Green's formula (see reference 3) we have formally

$$(\partial\varphi, \partial\varphi) + (\bar{\mathfrak{D}}\varphi, \bar{\mathfrak{D}}\varphi) = (\varphi, \Box\varphi) + \int_{bB} [\varphi\Lambda^*(\partial\varphi)^- - (\bar{\mathfrak{D}}\varphi)^-\Lambda^*\varphi] = 0$$

so $\partial\varphi = \bar{\mathfrak{D}}\varphi = 0$. In order to justify this conclusion, it is necessary to have further information concerning the boundary behavior of the form ϕ. However, an examination of the proof given in reference 3 for the existence of θ_p will show that in the uniqueness requirement (U) we may add the restriction that ϕ is of class C^1 in the closure of B. Under this additional restriction the above application of Green's formula is seen to be justified. Since $\bar{\Box}\varphi = \Box\varphi = 0$, we conclude in a similar fashion that $\bar{\partial}\varphi = \bar{\mathfrak{D}}\varphi = 0$ and thus

$$d\varphi = (\partial + \bar{\partial})\varphi, \ \delta\varphi = (\mathfrak{D} + \bar{\mathfrak{D}})\varphi = 0. \tag{3.2}$$

Now let S_ϵ be the set of points of the manifold whose distance from the boundary of B is less than ϵ. If ϵ is small enough, S_ϵ will possess a singularity $\theta_p(z, \zeta)$ for $\Box$ in terms of which

$$\frac{1}{2}\{\varphi(\zeta)\} = -\int_{C_\epsilon} [\varphi\Lambda^*(\partial\theta_p)^- - (\bar{\mathfrak{D}}\theta_p)^-\Lambda^*\varphi], \tag{3.3}$$

where $\{\varphi(\zeta)\}$ denotes $\phi(\zeta)$ if ζ is in $B \cap S_\epsilon$, the form 0 if ζ is outside $B \cap S_\epsilon$, and where C_ϵ is that portion of the boundary of S_ϵ which lies interior to B. This formula shows that $\{\varphi(\zeta)\}$ is a form of class C^1 in S_ϵ and hence φ defines a harmonic field satisfying (3.2) in $B \cup S_\epsilon$ provided that we set $\varphi = 0$ outside B.

Now let (ρ, σ) have one of the following four sets of values:

$$(\rho, 0), \ (k, \sigma), \ (0, \sigma), \ (\rho, k). \tag{3.4}$$

In the first two cases the equations (3.2) imply that ϕ and $*\phi$ respectively are complex-analytic in $z^1, z^2, \ldots, z^k$ while in the last two cases they imply that ϕ, $*\phi$ are complex-analytic in $\bar{z}^1, \bar{z}^2, \ldots, \bar{z}^k$. Hence if ϕ is of one of the types (3.4) and satisfies (3.1), then it vanishes identically. We have therefore established the following result:

A fundamental singularity $\theta_p(z, \zeta)$ exists on every proper compact submanifold of an arbitrary Kähler manifold if either one of the integers ρ, σ is equal to 0 or k.

4. Let $\varphi = \prod_{\rho,\sigma} \varphi$ satisfy

$$\bar{\partial}\varphi = \bar{\mathfrak{D}}\varphi = 0 \tag{4.1}$$

on a proper compact subdomain B_0 of the Kähler manifold, and let B be any subdomain of B_0 with regular boundary whose closure lies in the interior of B_0. If B possesses a singularity θ_p for $\Box = {}^1/_2 \prod_{\rho,\sigma} \Delta$ we have

(see reference 3)

$$\frac{1}{2}\{\varphi(\zeta)\} = -\int_{bB}[\varphi\Lambda * (\bar{\partial}\theta_p)^- - (\mathfrak{T}\theta_p)^-\Lambda^*\varphi]. \qquad (4.2)$$

This is a Cauchy's formula for fields ϕ on B_0 satisfying (4.1), the kernels being $\bar{\partial}\theta_p$ and $\mathfrak{T}\theta_p$.

We have seen that B_0 always possesses a singularity corresponding to complex-analytic forms of type $(p, 0)$, and formula (4.2) therefore provides a Cauchy representation for such forms in which $\mathfrak{T}\theta_p = 0$.

Since by (4.2) a closed Kähler manifold has a singularity θ_p for $\square = {}^1/_2 \prod_{p,\sigma} \Delta$ if and only if all forms satisfying (4.1) vanish identically, the restriction to submanifolds with boundary is obviously necessary.

* Apart from unimportant numerical factors. Dr. E. Calabi has recently called attention to a paper of Guggenheimer[4] in which some of the operators were defined on symplectic manifolds.

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*SPECTRAL SENSITIVITY OF THE CONES IN THE DARK ADAPTED HUMAN EYE**

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The rods of the human eye exist in great numbers in the peripheral retina, and the cones exist primarily, although not exclusively, in the centrally placed fovea. The cones mediate day (or bright light) vision and the rods, night (or dim light) vision.[9]

The two types of receptor exhibit different sensitivities to the wave-lengths of the visible spectrum. Sensitivity for a given wave-length, or luminosity, is defined as the reciprocal of the energy of that wave-length required to produce a constant visual effect. Rod visibility is maximum at 505 to 510 mμ[11, 22, 1, 18] and decreases toward the violet and red ends of the spectrum.

The cone luminosity curve has been determined by four methods classi-

fiable on the basis of the criterion of constant effect. The first method involves a direct brightness comparison of different wave-lengths against a constant standard.[3, 8, 18, 23] The second method makes use of the step-by-step (or small step) procedure, in which one wave-length is matched to a constant brightness with a neighboring wave-length, a third is matched with the second, etc.[12, 6, 4, 2] The third method involves an intensity adjustment to extinguish flicker. When two colors of different brightness are presented in rapid alternation, they flicker; when they stop flickering (due to proper adjustment of intensity) they are considered equally bright.[13, 15, 3, 17, 19, 14] The fourth method is characterized by the determination of thresholds.[22, 21]

In 1924 the International Commission on Illumination made a survey[16] of the existing luminosity data obtained by the first three methods and adopted a set of adjusted average values which have served as an international standard. The ICI curve is a smooth, symmetrical function and has a peak at 555 mμ. It represents cone function and is called the photopic curve.

In recent years some doubts have arisen concerning the detailed reliability of the ICI curve. On examination, the earlier curves of Gibson and Tyndall[6] and of Coblentz and Emerson[3] show considerable deviations from the standard in the blue end of the spectrum and seem to have a definite hump near 460 mμ. Recently Stiles[20] has reported a luminosity curve with a hump near 460 mμ. Wald[22] produced a cone sensitivity curve, much extended in spectral range, that also shows a hump near 460 mμ. According to Wald the change in curve direction near 460 mμ is due to absorption by the macula lutea (or yellow pigment) which covers the central fovea. Stiles[21] has published three individual cone luminosity curves but does not think that the effect at 460 mμ can be completely explained by macular absorption. An additional experiment[21] shows that the effect is accentuated at a location 5° from the center of the fovea.

Besides the abrupt change in the curve near 460 mμ, another one seems to exist near 600 mμ. Two of Stiles' three individual cone luminosity curves show a "depression" of the curve near 600 mμ while the third does not. Sloan's earlier data,[18] with low intensity and a small field (57 minutes), shows a similar effect, as do Granit's results[7] on the photopic dominator curves for the cat. Finally, results obtained by Walters and Wright in 1943[23] provide further evidence on the depression at 600 mμ. It is probable that the effect at 600 mμ cannot be understood in terms of absorption by the ocular media.

Walters and Wright have suggested that the red receptors of the Young-Helmholtz theory tend to maintain their sensitivity over a greater range of intensity than do the green and blue receptors; and Forbes[5] earlier made the same suggestion with respect to Sloan's data. Granit explains

the depression at 600 mμ for the cat's eye as due to a deficiency of red receptors. The red receptors raise the dominator curve in the orange-red region but are insufficient to provide high visibilities in the yellow-orange. Stiles is of the opinion that three maxima of luminosity curve are involved and perhaps represent the individual sensitivity curves of the three receptors.

Recently we have been investigating luminosity curves of color blind individuals. It became necessary, as part of this work, to determine the luminosity curve of the normal person for standardizing comparison purposes. The work is now going on, but because of the fact that our data on the normal person have considerable bearing on the general problem of the shape of the luminosity curve, we present some results here. Data on only one condition of observation are presented and it will remain for the future to vary other parameters.

We used the threshold method. Our test field was a circular one subtending a visual angle 42 minutes in diameter. The test field was viewed by the subject's dark adapted eye and appeared in the center of a fixation ring, i.e., a thin, dim ring of white light that ordinarily subtended a visual angle 3 degrees in diameter.

We were fortunate to have the double monochromator apparatus which Hecht, Shlaer and Pirenne used in their quantum study.[11] We used it, except for the fixation ring, in its original arrangement. Light from a ribbon filament tungsten source (run on 6 volts 18 amperes D.C.) passes through a set of filters and wedges (Jena glass type), is focused on the entrance slit of the first monochromator and then passes to the second monochromator (Schmidt and Haensch type) by way of a middle slit. On leaving the exit slit the light floods the field lens, which brings the rays to a focus at an artificial pupil (2.5 mm. diameter) thus providing Maxwellian view. The fixation ring is projected through a hole in the side of the viewing tube onto a piece of thin glass set at 45° to the line of sight to the test field and centered at a distance of one inch from the pupil. The subject sees the ring in the dark surround of the test field and in the same frontal plane. An electrically controlled shutter is interposed between the two monochromators; it presents the test field to the eye for 4 milliseconds at each exposure. The subject presents himself with a single exposure (when he has good fixation) by releasing a key.

We made new wave-length and energy calibrations of the apparatus. A constant wave-length band of 5 mμ throughout the spectrum was used in all determinations. The filters and wedges were measured for their selective transmission in a Beckman spectrophotometer, and the data were corrected accordingly. The energy measurements were done with a photronic cell (Weston, Model 594RR, at the pupil position) and a moving coil galvanometer. The spectral response of the photronic cell was twice calibrated by the Bureau of Standards in a period of 27 months.

At the beginning of each session of the subject became dark adapted for ten minutes, a sufficient time for the cones to gain full sensitivity. Then the spectral lights were presented to him by the method of limits; the threshold datum for each wave-length in any one session is the average for three ascending and three descending series that involved small steps of log energy. The subject responded by activating a bell to indicate whether or not he saw the light. Each sitting was restricted to an hour in order to minimize subject fatigue. A good deal of time was spent in making slit settings, and so only about eight wave-length positions could be covered in one sitting. Since determinations were made at nearly each 10 mμ in most regions of the spectrum between 415 and 760 mμ, and since for each position the threshold was repeated two, three or even more times, the production of a complete curve for a single subject involved the combined effort of the experimenter and subject for a considerable period of time.

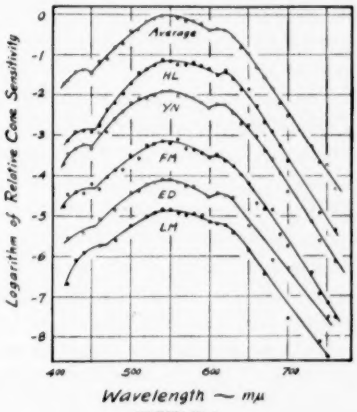

FIGURE 1

Logarithm of relative cone sensitivity as a function of wave-length. The stimulus is a centrally viewed round field, 42 minutes in diameter. It appears for a duration of 4 milliseconds. The topmost curve includes the averaged data for the five subjects whose individual data are given in the lower curves. Each successive curve is lowered through one logarithmic unit for clarity of presentation.

Each of our 5 subjects had normal color vision. The subjects were tested with the Stilling test and the Shlaer anomaloscope before they participated in the experiment. The ages of the subjects varied between 20 and 30. With the exception of LM, all were males.

Figure 1 gives the individual cone sensitivity curves for five subjects; the topmost curve presents the average data.† The basic data of these curves are relative energies required for the cones to respond to the spectral lights at the absolute threshold; the logarithms of the reciprocals of these values (i.e., log sensitivity values) are here plotted. The peak of the average curve is arbitrarily set at zero (i.e., maximum sensitivity is set at unity). For purposes of clarity of presentation each successive curve is moved downward through one logarithmic unit. It will be noticed that the individual curves do not have peaks at the value log *Sensitivity* = 0. Since the peak of the average curve is assigned the value of zero, data for a given individual are either higher or lower than the average. By this de-

vice an indication of deviations of threshold values from the average are obtained.

All of the curves of figure 1 show abrupt changes in direction near 460 mμ and 600 mμ; hence they support the results of other investigators who found similar effects. The effects seem to be characteristic of the cone sensitivity curve as obtained with a small field that is well within the dark adapted fovea.

The depression at 600 mμ is not as prominent in the average curve as it is in individual curves. In this connection it should be noted that subject HL probably shows the effect at a wave-length nearer 610 mμ than 600 mμ. The other subjects give data more nearly in accord with the averaged results.

The peaks of our individual curves are in the neighborhood of 550 mμ, or possibly slightly below. The peak of the ICI photopic luminosity curve is 555 mμ. Arndt[2] and Fedorov, *et al.*,[4] suggest that the peak should be around 565 mμ at high intensities. The latter authors found it to be near 558 mμ at a low intensity. Wald[22] estimates that it may occur at 562 mμ. Of the three individual curves reported by Stiles[21], probably none has its peak above 550 mμ. Walters and Wright [23] and Sloan[18] report that the peak is close to 540 mμ for low intensities. For a 20 minute area, Wright[24] found a peak near 560 mμ.

We have wondered whether fixation conditions might not have an effect on the position of the visibility peak. One of our subjects did, in fact, show a peak near 540 mμ when the 3° fixation ring measured. When the ring was reduced to 2° and slightly raised in brightness she showed a peak near 550 mμ. The other subjects all showed a peak near 550 mμ with the 3° ring.

Our curves exhibit higher luminosity values in the blue end of the spectrum than does the ICI standard. The difference between the log sensitivity values at 555 mμ and 415 mμ is, for our data, about 1.8 log units; for Wald's data, it is about 2.2 log units; and for Stiles' data it is about 1.7 log units. These values are to be contrasted with those of the ICI curve: 2.7 log units. As judged by the data of Wald, Stiles and ourselves, the ICI curve underestimates luminosity in the blue by a considerable amount.

It will certainly be important if further analysis indicates that the abrupt changes in curve direction found near 460 mμ and 600 mμ may be associated with three specifiable components. The relationship of such a finding to the Young-Helmholtz trichromatic theory is clear.

Summary.—New data on cone sensitivity, based on threshold measurements, are reported. Sensitivity is at a maximum near 550 mμ. The spectral sensitivity curve shows two points of abrupt change in direction: one near 460 mμ and the other near 600 mμ. These findings are in line with the results of some other experiments.[18, 21, 23]

* Prepared under Project No. NR 142-404, Contract N6onr-271, Task Order IX, between Columbia University and the Office of Naval Research, United States Navy. Reproduction in whole or in part permitted for any purpose of the United States Government.

† For the numerical data of the experiment, order Document 3423 from the American Documentation Institute, 1719 N St., N. W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film) or $1.00 for photocopies (6 × 8 in.) readable without optical aid.

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CONFIGURATIONS OF POLYPEPTIDE CHAINS WITH EQUIVALENT CIS AMIDE GROUPS

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In recent papers[1-3] we have pointed out that stable configurations of polypeptide chains must show the bond distances and bond angles found in simple peptides and related simple substances, that each amide group must be planar, with either the cis-configuration or the trans-configuration about the C′—N bond, and that the carbonyl and imino groups (except for proline or hydroxyproline residues) must be involved in the formation of hydrogen bonds, with N—H···O distance approximately 2.8 A, and with the oxygen atom nearly on the N—H axis. We have also presented evidence indicating that certain configurations around the two single bonds from the amide groups to the α carbon atom are energetically favored, and have discussed the 36 polypeptide-chain structures involving equivalent trans-amide groups with one or another of six favored azimuthal orientations about the N—C single bond and also one of six favored orientations about the C—C′ single bond.[4] In the present paper we discuss the 36 configurations of equivalent cis-amide groups corresponding to these favored orientations, and also a few other configurations of cis-amide groups.

In the study of these structures we have been greatly aided by the use of a special set of molecular models, constructed of wood on the scale 2.5 cm. = 1 A. The wooden pieces representing the atoms are joined together by the use of steel pins to form a model of a polypeptide chain, and the models are fitted with screw clamps on the steel pins which permit the orientations around the bonds to be fixed. The model representing the hydrogen atom of the imino group is cupped in such a way that the model representing the oxygen atom may fit into it, at a distance between N and O corresponding to 2.8 A. The position of the cup is adjustable, to allow a bend in the hydrogen bond of as great as 20°. The models conform to the accepted van der Waals radii of the atoms, permitting the easy recognition of serious steric hindrance for a proposed configuration. A detailed description of these molecular models will be published later.

The favored orientations about the N—C bond (the bond between the nitrogen atom of an amide group and the adjacent α carbon atom) are those in which one of the three other bonds formed by the α carbon is either cis or trans to the N—H bond. These orientations can be described by an azimuthal angle φ, as follows: $\varphi = 0°$, CC′ cis to NH; $\varphi = 60°$, CH trans to NH; $\varphi = 120°$, CR cis to NH; $\varphi = 180°$, CC′ trans to NH:

$\varphi = 240°$, CH cis to NH; $\varphi = 300°$, CR trans to NH. The similar favored orientations about the C—C′ bond can be described by an azimuthal angle χ, as follows: $\chi = 0°$, CN cis to CO; $\chi = 60°$, CH trans to CO; $\chi = 120°$, CR cis to CO; $\chi = 180°$, CN trans to CO; $\chi = 240°$, CH cis to CO; $\chi = 300°$, CR trans to CO.

The 36 configurations are described in table 1. In general they are helical structures; in a few cases the helix has degenerated to a ring. Many of the structures are eliminated by serious steric hindrance. Of those that are not thus eliminated, some cannot enter into hydrogen-bond formation with adjacent similar polypeptide chains, and are eliminated for this reason. No configurations with intramolecular hydrogen bonds are contained in this set. For each of the configurations that is not eliminated by steric hindrance, the pitch of the helix (axial length per turn), the number of residues per turn, the approximate orientation of the CO and NH groups relative to the helical axis, and the suitability of the structure to form hydrogen bonds are given in the table. A helical molecule can form a crystal in which it is linked to equivalent molecules by equivalent hydrogen bonds only if it has a twofold, threefold, fourfold, or sixfold axis of symmetry.

Discussion of the Structures.—No helical structures suitable for proteins or polypeptides in general have been discovered by the foregoing considerations. The most interesting of the helical structures described in table 1 is number 16, with three residues per turn. These helical molecules could form hydrogen bonds with similar helixes arranged in a hexagonal lattice. The dimensions of the unit are such as to make the structure a possible one for polyalanine, but not for other polypeptides or for proteins. Structures 4 and 5, with four residues per turn, could form hydrogen-bonded tetragonal crystals, but are probably eliminated by steric hindrance. Similarly, structures 28 and 29, with three residues per turn, could form hexagonal crystals, but also are probably eliminated by steric hindrance. The helixes 24 and 35, with 4.5 residues per turn, are interesting as configurations for the polypeptide chain, but are unable to form equivalent hydrogen bonds with adjacent chains because of the absence of a crystallographic symmetry element.

We might ask whether satisfactory helical configurations of polypeptide chains with equivalent cis-amide groups could be formed by permitting orientations around the single bonds to the α carbon atom other than the postulated favored orientations. No configurations of this sort involving intramolecular hydrogen bonds exist. In order to form intermolecular hydrogen bonds between equivalent amide groups the helix would have to have a twofold, threefold, fourfold, or sixfold screw axis of symmetry. Configurations with threefold and fourfold screw axes are described in table 1, and have been discussed in the preceding paragraph. The geometry of the

TABLE 1

CONFIGURATIONS OF EQUIVALENT cis-AMIDE GROUPS WITH FAVORED ORIENTATIONS ABOUT C—N AND C′—C BONDS[a]

A. Trans-trans Configurations:

1. CH trans to NH, CH trans to CO; $\varphi = 60°$, $\chi = 60°$. A four-residue puckered ring is formed, but the hydrogen atoms attached to the α carbon atom are at the corners of a square only about 1.4 A on edge. The consequent steric hindrance is probably so great as to eliminate this structure. The carbonyl and imino groups point out and up, and hydrogen bonded tetragonal layers could be formed, with $a = 9.6$ A (the unit containing two cyclic tetrapeptide molecules), and with c determined by the nature of the side chains.

2. CR trans to NH, CR trans to CO; $\varphi = 300°$, $\chi = 300°$. A four-residue puckered ring, resembling 1. The four R groups are crowded together, and strong steric hindrance eliminates this structure.

3. CC′ trans to NH, CN trans to CO; $\varphi = 180°$, $\chi = 180°$. A planar cyclic dipeptide. This is the diketopiperazine structure. The molecules can form long strings held together by hydrogen bonds, as in the diketopiperazine crystal.[5]

4. CH trans to NH, CR trans to CO; $\varphi = 60°$, $\chi = 300°$. Helix with pitch 11.8 A, four residues per turn. Amide groups in vertical plane, with NH and CO projecting out diagonally. Each amide group could form two hydrogen bonds with a corresponding amide group of a similar chain, with reversed orientation, giving a tetragonal crystal, with $a = 7.5$ A, $c = 11.8$ A, eight residues per unit. However, an H atom and an R group on α carbon atoms adjacent to each amide group are directed toward one another, and steric hindrance eliminates this structure for all R groups except H. The structure probably could hence be assumed only by polyglycine; but for polyglycine the density with this structure would be 1.15 g. cm.$^{-3}$, which is too small. The structure would hence not permit maximum stabilization for polyglycine by electronic van der Waals attraction, and it is accordingly eliminated.

5. CR trans to NH, CH trans to CO; $\varphi = 300°$, $\chi = 60°$. Four-residue helix with pitch 11.8 A, resembling 4, and probably eliminated for the same reasons.

6. CH trans to NH, CN trans to CO; $\varphi = 60°$, $\chi = 180°$. Ruled out by steric hindrance of C and H of NH.

7. CR trans to NH, CN trans to CO; $\varphi = 300°$, $\chi = 180°$. Ruled out by steric hindrance of N and C.

8. CC′ trans to NH, CH trans to CO; $\varphi = 180°$, $\chi = 60°$. Ruled out by steric hindrance of H (of CH) and C′O.

9. CC′ trans to NH, CR trans to CO; $\varphi = 180°$, $\chi = 300°$. Ruled out by steric hindrance of αC and O.

B. Cis-cis Configurations:

10. CH cis to NH, CH cis to CO; $\varphi = 240°$, $\chi = 240°$. Ruled out by steric hindrance of C and C.

11. CR cis to NH, CR cis to CO; $\varphi = 120°$, $\chi = 120°$. Ruled out by great steric hindrance.

12. CC′ cis to NH, CN cis to CO; $\varphi = 0°$, $\chi = 0°$. A nearly strainless planar ring containing seven residues. Some steric hindrance may occur between R groups, which are all on one side of the plane, with the H atoms on the other side. The CO and NH groups could form intermolecular hydrogen bonds with equivalent molecules, except that the sevenfold axis prevents the formation of a crystal containing only equivalent amide groups.

TABLE 1—*Continued*

13. CH cis to NH, CR cis to CO; $\varphi = 240°$, $\chi = 120°$. Ruled out by steric hindrance of R and NH.

14. CR cis to NH, CH cis to CO; $\varphi = 120°$, $\chi = 240°$. Ruled out by steric hindrance of H of CH and H of NH.

15. CH cis to NH, CN cis to CO; $\varphi = 240°$, $\chi = 0°$. Tight helix with pitch 6.6 A, three residues per turn. Ruled out by steric hindrance of R and CO of next turn of the helix.

16. CR cis to NH, CN cis to CO; $\varphi = 120°$, $\chi = 0°$. A tight helix with pitch 6.6 A and three residues per turn, resembling 15. Close contact of CH and CO of next turn, but probably not enough to eliminate the structure. NH points out laterally, CO out and down by 60°. A hexagonal lattice could be formed, each chain being surrounded by six parallel chains, with three of which it forms hydrogen bonds using its NH groups, and with the other three hydrogen bonds using its CO groups. The hexagonal unit has $a = 6.7$ A, $c = 6.6$ A, three residues per unit. The assumed density 1.40 g. cm.$^{-3}$ corresponds to residue weight 72. That of alanine is 71; accordingly only polyalanine might be expected to have this structure.

17. CC′ cis to NH, CH cis to CO; $\varphi = 0°$, $\chi = 240°$. Helix with pitch 7.1 A, three residues per turn. Ruled out by steric hindrance of R and H of NH, and by inhibition of hydrogen-bond formation between chains through steric hindrance between H of CH and O of adjacent chain (see 18).

18. CC′ cis to NH, CR cis to CO; $\varphi = 0°$, $\chi = 120°$. Helix with pitch 7.1 A, three residues per turn, resembling 17. H of CH in contact with H of NH. The NH groups point closely toward R of CHR separated from it by two residues: R shields NH so that no satisfactory hydrogen bond could be formed; hence the structure is ruled out.

C. Trans-cis Configurations:

19. CH trans to NH, CH cis to CO; $\varphi = 60°$, $\chi = 240°$. Ruled out by steric hindrance of H of CH and H of NH.

20. CR trans to NH, CR cis to CO; $\varphi = 300°$, $\chi = 120°$. Helix with pitch 7.8 A, three residues per turn. CO points out, NH points out and up. NH is close to axis of helix and is in steric interference with adjacent H and R groups, serious enough to eliminate the structure.

21. CC′ trans to NH, CN cis to CO; $\varphi = 180°$, $\chi = 0°$. Ruled out by great steric hindrance.

22. CH trans to NH, CR cis to CO; $\varphi = 60°$, $\chi = 120°$. Ruled out by great steric hindrance.

23. CR trans to NH, CH cis to CO; $\varphi = 200°$, $\chi = 240°$. Ruled out by steric hindrance of R and NH.

24. CH trans to NH, CN cis to CO; $\varphi = 60°$, $\chi = 0°$. Open helix with pitch 8.1 A, 4.5 residues per turn. H of CH in tight helix, in contact with equivalent adjacent atoms. NH points out laterally, CO out and down at 45°. Not suited to formation of intermolecular hydrogen bonds with equivalent molecules.

25. CR trans to NH, CN cis to CO; $\varphi = 300°$, $\chi = 0°$. Open helix with pitch 8.1 A, 4 5 residues per turn, resembling 24, but with H and R interchanged. Ruled out by steric hindrance of R and R (R—R = 1.8 A).

26. CC′ trans to NH, CH cis to CO; $\varphi = 180°$, $\chi = 240°$. Ruled out by great steric hindrance.

TABLE 1—*Continued*

27. CC′ trans to NH, CR cis to CO; $\varphi = 180°$, $\chi = 120°$. Ruled out by great steric hindrance.

D. Cis-trans Configurations:

28. CH cis to NH, CH trans to CO; $\varphi = 240°$, $\chi = 60°$. Helix with pitch 8.5 A, three residues per turn. Intermolecular hydrogen bonds can be formed with three adjacent chains, oppositely directed. Hexagonal crystal with $a = 9.7$ A, $c = 8.5$ A, containing six residues, with average residue weight about 97; calculated density then 1.35 g. cm.$^{-3}$. Probably eliminated by steric hindrance of H of CH, O, and R.

29. CR cis to NH, CR trans to CO; $\varphi = 120°$, $\chi = 300°$. Helix with pitch 8.5 A, three residues per turn, resembling 28. Probably eliminated by steric hindrance between R, O, and H of CH.

30. CC′ cis to NH, CN trans to CO; $\varphi = 0°$, $\chi = 180°$. Ruled out by great steric hindrance of αC and H of NH.

31. CH cis to NH, CR trans to CO; $\varphi = 240°$, $\chi = 300°$. Tight helix with 2.6 residues per turn. Ruled out by steric hindrance of R, αC, and N.

32. CR cis to NH, CH trans to CO; $\varphi = 120°$, $\chi = 60°$. Ruled out by steric hindrance of CH and CO.

33. CH cis to NH, CN trans to CO; $\varphi = 240°$, $\chi = 180°$. Ruled out by steric hindrance of C and C′.

34. CR cis to NH, CN trans to CO; $\varphi = 120°$, $\chi = 180°$. Ruled out by great steric hindrance of αC and NC′.

35. CC′ cis to NH, CH trans to CO; $\varphi = 0°$, $\chi = 60°$. Open helix with pitch 8.5 A, 4.5 residues per turn, resembling 24, but with CO and NH interchanged. Ruled out because of inability to form a hydrogen-bonded structure.

36. CC′ cis to NH, CR trans to CO; $\varphi = 0°$, $\chi = 300°$. Open helix with pitch 8.1 A, 4.7 residues per turn, resembling 24. CO points out laterally, NH diagonally. R groups are on the axis; hence the configuration is ruled out by steric hindrance (R—R = 1.7 A).

[a] The values of φ and χ for structures 1 to 36 in this table apply also to structures 1 to 36 in the corresponding table for trans-amide groups.[4]

cis-amide group is such that no acceptable configuration of equivalent residues with a twofold screw axis can be constructed. Helical arrangements of polypeptide chains of equivalent cis-amide groups with a sixfold screw axis can be built in many closely similar ways. Thus the orientations $\varphi = 30°$ and $\chi = 355°$ lead to a helix with six residues per turn and pitch 8.6 A. This helix can form hydrogen bonds with equivalent adjacent helixes to give a hexagonal crystal with $a = 10.8$ A and $c = 8.6$ A. The CHR groups are near the helical axis of each molecule, the hydrogen atoms being in contact with their centers at the distance 1.5 A from the axis. The β carbon atoms of the side chains are at radius 2.6 A. Each chain is linked to each of the six adjacent chains by a pair of hydrogen bonds formed by an HNCHRCO group. With assumed density 1.35 g. cm.$^{-3}$, the mean residue weight is 11.8. This structure is accordingly a possible one, though perhaps unlikely, for polypeptides or proteins with average residue weight

about 118. It is interesting to note that two helixes of this sort could be intertwined, without evident steric hindrance, in such a way as to lead to a hexagonal crystal with $a = 10.8$ A and $c = 4.3$ A, the latter identity distance corresponding to the translation from an equivalent point on one to a similar point on the other helix intertwined with it. The residue weight for this structure is calculated to be about 60, and accordingly it is a possible structure for polyglycine.

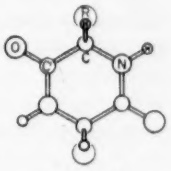

FIGURE 1

A drawing of the planar cyclic dipeptide diketopiperazine, structure 3 of table 1.

Helixes with six residues per turn are also obtained by other pairs of orientations around the C—N and C—C bonds. For example, the orientations $\varphi = 22°$, $\chi = 338°$ lead to a somewhat similar six-residue helix, with pitch 11.7 A. It is possible that three helixes of this sort could be intertwined, giving molecules that could form crystals with a hexagonal unit with $a = 10.8$ A and $c = 3.9$ A, containing six residues, with residue weight about 54. This three-chain structure is accordingly also a possible one for polyglycine. We consider it to be unlikely, however, because there is probable instability connected with the cis configurations of the amide groups, rather than the trans configuration, and also instability connected with the orientations around the N—C′ and C—C′ bonds.

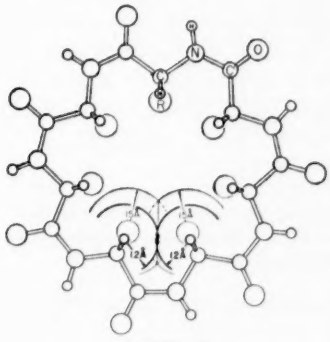

FIGURE 2

A drawing of a possible structure of a cyclic heptapeptide, structure 12 of table 1.

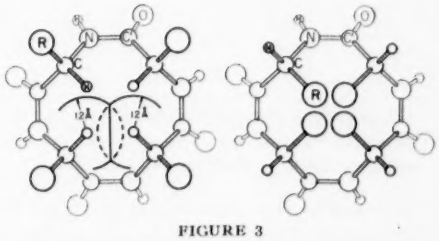

FIGURE 3

A drawing of two structures for cyclic tetrapeptides, structures 1 and 2 of table 1. Structure 2 is ruled out, except for tetraglycyl, by steric hindrance between R groups.

There are four cyclic structures described in table 1. Of these the only one that is known to be represented in nature is the dipeptide ring, structure 3, which is the diketopiperazine structure (Fig. 1). The planar seven-residue ring, structure 12, may well be shown by cyclic heptapeptides,

despite its inability to form hydrogen bonds with similar molecules (Fig. 2). Presumably heptapeptides crystallized from a solvent would tend to include molecules of the solvent in the crystals. The other two cyclic peptides described in table 1 are the cyclic tetrapeptides, structures 1 and 2 (Fig. 3). Structure 2 is ruled out, except for tetraglycyl, by steric hindrance between R groups. Structure 1 would presumably be assumed by cyclic tetrapeptides; some instability is conferred on it by the close approach of hydrogen atoms. Except for this instability, the tetragonal crystals involving staggered sheets of the cyclic tetrapeptide molecule connected by hydrogen bonds would be predicted for all cyclic tetrapeptides, the space

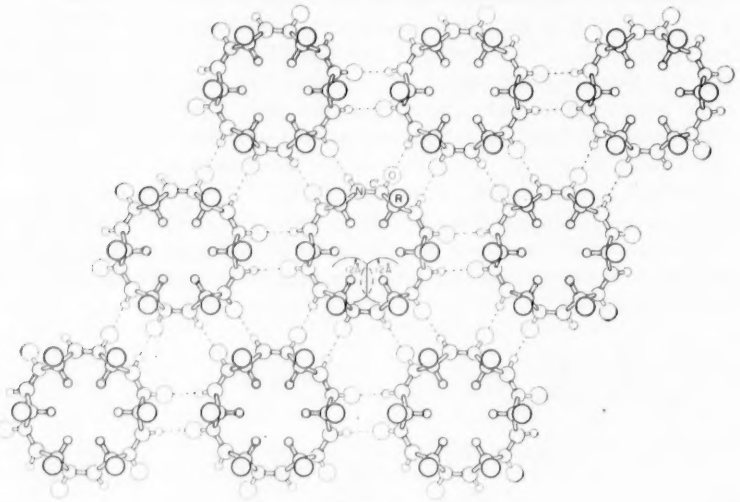

FIGURE 4

A drawing of a possible structure of a cyclic hexapeptide with unfavorable orientations about the bonds to the α carbon atom. Possible hydrogen bonding is shown between adjacent molecules, forming a two-dimensional crystalline sheet.

occupied by side chains being provided by suitable selection of the value of the length of the c axis of the tetragonal unit.

In addition, there are six cyclic structures involving equivalent cis-amide groups with unfavorable orientations about the bonds to the α carbon atom. These are two tripeptide structures, two pentapeptide structures, and two hexapeptide structures, all similar to the tetrapeptide structures 1 and 2. The three structures analogous to structure 2, with the R groups pointing toward the symmetry axis, are eliminated by steric hindrance. The other three structures involve a tight fit of the hydrogen atoms, which would confer some instability upon them. The pentapep-

tide cannot form hydrogen-bonded crystals with all residues equivalent, but the tripeptide can form crystals of this sort. In the cyclic tripeptide molecules the orientation about the N—C axis is $\varphi = 280°$, and that about the C—C′ axis is $\chi = 283°$. Hexagonal crystals involving staggered layers can be formed by the cyclic tripeptide molecules, with two slightly different kinds of molecules alternating. Those of one kind form pairs of hydrogen bonds with their three neighbors with use of CONH groups in each pair, whereas the pairs of hydrogen bonds are formed by the molecules of the other kind with use of NHCHRCO groups. The lateral edge of the hexagonal unit is $a = 10.4$ A, the value of c being determined by the side chains. For cyclic triglycyl, for which mirror-image three-residue rings may be formed, the staggered sheet could be built up of equivalent residues, the pairs of hydrogen bonds being formed by the groups CONH of each molecule, or perhaps better by the groups NHCHRCO.

The cyclic hexapeptide with CH groups turned toward the hexagonal axis of the ring has orientations $\varphi = 337°$, $\chi = 337°$. The hydrogen atoms are about 1.7 A apart, introducing a slight instability due to steric hindrance. The molecules could form a hexagonal polar sheet with similar molecules, the hydrogen bonds being bent about 20° from the N—H axis. The value of a is 10.8 A, that of c being determined by the nature of the side chains. The hydrogen bonds are probably of the NHCHRCO type, a pair of bonds formed by this group in one molecule connecting it with each of the six surrounding molecules in the sheet, as shown in figure 4. No cyclic hexapeptide has yet been subjected to X-ray investigation.

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